Thesis submitted in partial fulfilment for the requirements of a PhD degree

C-Alkyl inositol phosphates for use in receptor-ligand engineering

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Declaration

The work described in this thesis was carried out at Imperial College Department of Chemistry, South Kensington between October 2005 and September 2008. All experiments and results reported are my own work, unless stated to the contrary.

Abstract

The phosphatidylinositol phosphates (PIP_ns) and inositol phosphates (IP_ns) are intricately involved in cell signalling. They bind to a vast array of proteins, which results in a host of physiological responses. Therefore it is difficult to determine the precise downstream effects of an individual protein-phosphoinositide interaction. Perturbations of these networks occur in pathological conditions such as cancer and diabetes increasing the need to understand these systems. Receptor-Ligand Engineering (RLE) may provide the tools to map these interactions. Chemical modification of the small PIP_n or IP_n ligand and complementary mutation of binding site amino acids is used to create a unique protein-ligand binding pair. Once the modified protein is engineered into the cell line, the dose dependent effects of its stimulation with the complementary ligand can be studied in isolation from signal pathway cross-talk.

Phosphatidylinositol 4,5-diphosphate [PtdIns(4,5)P_2 / PIP_2] and phosphatidylinositol 3,4,5-triphosphate [PtdIns(3,4,5)P_3 / PIP_3] analogues with *C*-alkyl groups replacing the axial inositol C-H protons would be suitable ligands for RLE. To date, no such analogues are known in the literature. The key challenges in preparation of such compounds are selective protection and deprotection of the *myo*-inositol hydroxyls, introduction of new inositol *C*-substituents with retention of *myo*-stereochemistry, and phosphorylation of an unnatural tertiary centre.

4-*C*-Alkyl IP_3 and 4-*C*-alkyl IP_4 analogues were chosen as targets to explore the chemical limitations of analogue synthesis. Orthoesters simultaneously tied up the 1-, 3- and 5-*O* differentiating between the remaining three hydroxyls in a rigid structure. Oxidation of the isolated 4-*O*H to the inos-4-ose and selective reintroduction of the *myo*-geometry by addition of dimethyl sulfoxonium methylide generated the key *exo*-methylene oxide intermediate. Lithium alkyl cyano cuprates were employed to open the *exo*-methylene oxide introducing primary, β-secondary, and β-tertiary alkyl and aryl protrusions.

4-*C*-Alkyl triols were prepared by regiocontrolled DIBAL-H reduction of the orthobenzoate to a benzyl ether, directed by the 4-*C*-alkyl protrusion. The corresponding 4-*C*-alkyl tetrols were obtained by acidic hydrolysis of the orthobenzoate and cleavage of the resultant benzoate ester. All polyols were then phosphorylated and globally deprotected to generate the final series of 4-*C*-alkyl IP_3 and IP_4 analogues.

Some initial investigations were also performed to extend this methodology to prepare 4- and 5-*C*-alkyl derivatives from a common precursor.

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Abbreviations

ATP	adenosine triphosphate
Bn	benzyl
^{t}Bu	*tert*-butyl
Bz	benzoyl
COSY	correlation spectroscopy
DAG	diacyl glycerol
DIBAL-H	diisobutylaluminium hydride
DMF	dimethylfomamide
DMP	Dess Martin periodinane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EF-Tu	elongation factor Tu
ENTH	epsin N-terminal homology
eq.	equivalents
ER	endoplasmic reticulum
FYVE	Fab1p, YOTB, Vac1 and EEA1
GDP	guanosine diphosphate
GPCR	G-protein coupled receptor
GTP	guanosine triphosphate
h	hours
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
ITC	isothermal titration calorimetry
IP_n	inositol polyphosphate
IR	infra-red
J	coupling constant
*m*CPBA	*meta*-chloroperbenzoic acid
min	minutes
MS	mass spectrometry
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
PDK-1	3-phosphoinositide dependent protein kinase 1
PG	protecting group
PH	plextrin homology
PI3K	phosphoinositide 3-kinase

PIP_n	phosphatidyl inositol polyphosphate
PITP	phosphatidyl inositol transport protein
PKB	protein kinase B
PKC	protein kinase C
PLC	phospholipase C
Pmb	*para*-methoxy benzyl
ppm	parts per million
PtdIns	phosphatidyl inositol
PtdOH	phosphatidic acid
PTEN	phosphatase and tensin homologue deleted on chromosome 10
PX	PhoX
Py	pyridine
R_f	retention factor
RLE	receptor-ligand engineering
RMT1	arginine methyl transferase 1
RNA	ribonucleic acid
rt	room temperature
SAH	*S*-adenosylhomocysteine
SAM	*S*-adenosylmethionine
SHIP	SH2 domain containing inositol-5-phosphatase
TBAF	*tert*-butyl ammonium fluoride
Tbdms	*tert*-butyldimethylsilyl
Tbdps	*tert*-butyldiphenylsilyl
TFA	trifluoroacetic acid
TfOH	trifluoromethane sulfonic (triflic) acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Tms	trimethylsilyl
TSA	*para*-toluene sulfonic acid
UV	ultra violet
VT	variable temperature
WT	wild type
XTP	xanthine triphosphate
δ	chemical shift

Biological Glossary

agonist	a ligand or drug which binds to and activates a receptor
antagonist	a ligand or drug which binds to a receptor and blocks receptor activity
eukaryotic	a cell with a high degree of internal organisation, including a nucleus which houses the DNA or genes
kinase	an enzyme which transfers a phosphate group to a molecule
K_M	the concentration of substrate which permits the enzyme to achieve half V_{max}
phosphatase	an enzyme which removes a phosphate group
proteome	the complete set of proteins expressed by a cell, tissue or organism
V_{max}	the rate of reaction when the enzyme is saturated with substrate, and therefore the maximum rate of reaction

Preface

This thesis is divided into seven main chapters. The first chapter introduces the biological background of this project and the long term goals, of which this work forms a key part at the very beginning. The second chapter introduces the chemical background on which this work was based. By reviewing the available literature, a synthesis strategy was designed in order to ensure that the available time was spent resolving the key transformations rather than in protecting group manipulations.

Chapters three to six contain the results and discussion. The primary aim of this thesis was to prove that the chemistry needed to create the target compounds was feasible, as without it, the long term goals of the project are impossible. Chapter three discusses the introduction of a 4-*C*-alkyl group onto the *myo*-inositol ring, followed by manipulation of the protecting groups to generate triols and tetrols in chapter four, which are phosphorylated and deprotected in chapter five. Throughout these chapters, the boundaries and chemical limitations of the synthesis are explored, generating valuable information which can be applied to the design of later analogues.

Chapter six introduces a more general synthetic design, conceived during this project, for the introduction of 4-, 5- and 6-*C*-alkyl groups from a common intermediate. Based on the results of the earlier experimental work and with the longer term goal of generating multiple *C*-alkyl groups at any ring position, this chapter stands alone, and makes an initial foray into demonstrating the feasibility of the suggested synthetic strategy.

Finally chapter seven contains the experimental procedures and spectral analysis for all the compounds prepared in chapters three to six.

Part of the work contained within this thesis is currently in press, having been accepted for publication by *Organic and Biomolecular Chemistry*. J. M. Swarbrick, S. Cooper, G. Bultynck, P. R. J. Gaffney; Regioselective deprotection of orthobenzoates for the synthesis of inositol phosphates.

Introduction

A relatively small number of interconvertable inositol polyphosphates (IP_n) and phosphatidylinositol polyphosphates (PIP_n) interact with a battery of proteins, leading to widespread and, in many cases, interlinked functions and effects.[1-5] These interactions are critical in essential cell processes such as growth, communication and differentiation. For example, generation of inositol 1,4,5-triphosphate stimulates calcium release which effects muscle contraction and vision;[5] PIP_ns are implicated in insulin signal transduction (dysfunction of which can lead to diabetes[4]) and growth factor pathways (where abnormal growth causes cancer[6]).

There is still much to learn about these species and their complicated yet specific interactions. Understanding of such signalling cascades would be immensely improved by a pharmacological map of exactly how they function. However, changes in signalling lipids are often masked by the large mass of steady-state structural lipids,[6] which has made studying the dynamics of the IP_n and PIP_n binding proteins in their native environment difficult.[2] Many studies of the roles of phosphoinositides in the cellular environment have relied on indirect or long-term methods of manipulating phosphoinositide levels (such as genetic knock-down, or overexpression of phosphoinositide metabolizing enzymes).[2] The ability of these systems to change over time makes the results of these studies difficult to interpret.[7] In many cases it has not been possible to determine precisely which IP_n- or PIP_n-protein interaction or sequence of interactions is responsible for a downstream effect. Fast-acting acute chemical inhibitors of phosphoinositide metabolising enzymes and agonists for particular phosphoinositide-binding proteins[2] are required to help elucidate these pathways on the timescale in which they function.

The synthetic chemistry described in this thesis prepares small molecule tools which could be used to study these systems. Using these ligands it is postulated that, combined with engineering of a protein's binding site, the precise effect of an individual lipid-binding protein interaction can be studied. The implications of being able to stimulate a protein of interest for unique activation by a small molecule at our discretion are very wide ranging. In the short term, it would allow us to assign the downstream effects of the protein under study unambiguously and in the long term it may suggest targets for the design of therapeutics which may benefit human health.

Chapter 1: The phosphoinositides and inositol phosphates as second messengers

This chapter provides an introduction to the phosphoinositides (IP_n and PIP_n). The first sections introduce the structure and numbering of these species and give a brief review of the historical events which have led to the widespread research interest and the need to understand this complex system. The second half of the chapter introduces receptor-ligand engineering, and its proposed application to interrogate the phosphoinositide signalling network.

In the 1950's Lowell and Mabel Hokin demonstrated that acetylcholine stimulated the turnover of inositol-containing phospholipids in cells.[8] This seminal discovery, termed the 'phospholipid effect', spiked a wave of biological research which has begun to uncover the complex phosphatidylinositol polyphosphate (PIP_n) and inositol polyphosphate (IP_n) cellular networks. At the current time, there are 7 known PIP_n and over 30 known IP_n in eukaryotic cells,[9] which are interconverted by a battery of kinases and phosphatases. These species are intricately involved in a multitude of cellular processes.[2,3,6,10,11]

1.1 Structure and numbering

The inositol polyphosphates (IP_ns) and phosphatidylinositol polyphosphates (PIP_ns) are closely related in structure. To clarify which species is being considered, it is helpful to introduce the terminology used for their structure and numbering.

The IP_ns are composed of the cyclohexane hexitol *myo*-inositol, and one or more phosphate esters, Figure 1.1. They are water soluble, polar molecules which are located in the cytosol.[13]

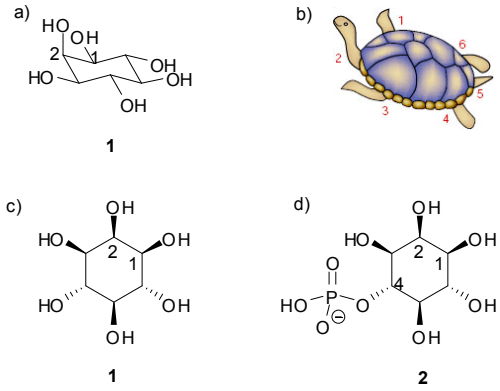


Figure 1.1: a) and c) *myo*-inositol; b) Agranoff's Turtle[12] d) inositol-4-phosphate

Agranoff's turtle mnemonic,[12] Figure 1.1b, illustrates and resolves much of the confusion that has surrounded inositol polyphosphate nomenclature. It demonstrates the position of the six

myo-inositol hydroxyl groups in the most thermodynamically stable chair conformation; the turtle's head represents the axial 2-hydroxyl and the four flippers and tail the five equatorial hydroxyls. The ring is conventionally numbered in an anticlockwise direction, when viewed from above, starting from the right front flipper, Figure 1.1c. *myo*-Inositol has *meso*-symmetry, with a mirror plane running through the axial 2-*O*H and equatorial 5-*O*H. Therefore, addition of a substituent to the 1-, 3-, 4- or 6-*O*H, which are not on the mirror plane, generates a chiral molecule. Usually only one enantiomer of a natural IP_n or PIP_n is bio-active. The standard chiral stereospecific numbering system is always referred to the D enantiomer (the right handed turtle). Any one or combination of the six ring positions can be reversibly phosphorylated, for example phosphorylation on the 4-*O*H would generate *myo*-inositol 4-phosphate, Ins(4)P, and its enantiomer *myo*-inositol 6-phosphate, Ins(6)P, Figure 1.1d.

The PIP_ns ($PtdInsP_n$, phosphatidylinositol polyphosphate, an inositol lipid) contain an additional hydrophobic component, usually phosphatidic acid (PtdOH, diacylglycerol phosphate), that is attached to the inositol 1-*O* via a phosphodiester link,[5] Figure 1.2.

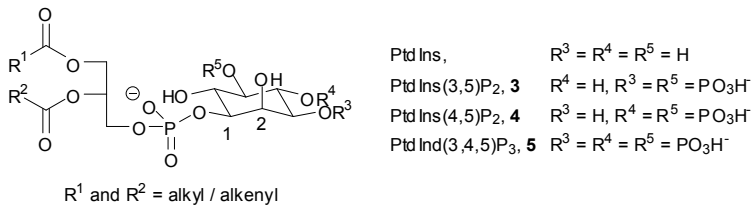


Figure 1.2: The structure of the phosphatidylinositol polyphosphates (PIP_ns)

PtdOH is composed of diacylglycerol (DAG) phosphate with two lipophilic fatty acid ester chains which may be saturated or (poly)unsaturated, R^1 and R^2, Figure 1.2; notably, most mammalian PIP_ns are characteristically polyunsaturated on the *sn*-2 fatty acid with 3 to 6 double bonds in the chain. Phosphatidylinositol is phosphorylated under physiological conditions at any of the 3-, 4- or 5-*O*H by specific kinases to prepare higher phosphatidylinositol polyphosphates (PIP_ns where n = no. of phosphates) such as phosphatidylinositol 3,5-diphosphate $PtdIns(3,5)P_2$, phosphatidylinositol 4,5-diphosphate $PtdIns(4,5)P_2$ and phosphatidylinositol 3,4,5-triphosphate $PtdIns(3,4,5)P_3$,[1] Figure 1.2. PIP_ns are amphiphilic molecules, located with their fatty alkyl chains in the hydrophobic cell membrane and polar inositol head groups in the cytosol.

1.2 Second messenger signalling

For a cell to react to an external signal, the signal must first be detected and transmitted across the physical boundary of the cell membrane.[14] Unlike lipophilic steroids, which can cross the fatty plasma membrane by passive diffusion, many chemical signals including growth factors and hormones are prevented from entering the cell by its hydrophobic nature. Instead, these primary messengers bind to one of three types of cell surface receptors situated in the membrane;

ion channels, tyrosine kinase receptors or G-protein coupled receptors (GPCRs).[15] Ion channels in the membrane are usually closed and open on receptor activation, allowing ions such as Na^+ and K^+ to flow into or out of the cell. Activation of tyrosine kinase receptors causes autophosphorylation of one or more tyrosine residues on the cytoplasmic domain of the receptor, which recruits regulatory adaptor proteins. Activation of a transmembrane GPCR causes exchange of GDP for GTP in the α-subunit of an associated heterotrimeric G-protein, which allows the G-protein to dissociate into the monomeric Gα.GTP subunit and a βγ dimer. Therefore, the arrival of a primary messenger, and binding to one of these cell surface receptors ultimately activates an enzyme, which may be either a cytosolic domain of the receptor or may occur indirectly. This enzyme then acts on a second messenger precursor (such as a PIP_n) in the vicinity of the receptor.[15] In the case of phosphoinositide signalling this activation pathway generates either a water soluble (IP_{n+1}) or a membrane bound (PIP_{n+1}) second messenger which exert numerous downstream effects on the cell. These, usually very transient and tightly regulated, second messengers go on to activate their targets, resulting in a downstream response.[14] This mechanism allows second messengers to be rapidly generated in response to extracellular stimuli.

The discovery of the PIP_ns began with the separation and characterisation of the cellular inositol-containing phospholipids, which demonstrated that three individual species were present; phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate [PtdIns(4)P] and phosphatidylinositol 4,5-diphosphate [PtdIns(4,5)P_2].[16] PtdIns is formed from PtdOH and *myo*-inositol in the endoplasmic reticulum (ER) and transported to the plasma membrane by a specific carrier protein (PtdIns Transport Protein, PITP). All three species are thought to be maintained at a steady-state level in the inner leaflet of the plasma membrane by a dynamic equilibrium between enzymatic phosphorylation and dephosphorylation,[10,11] Figure 1.3.

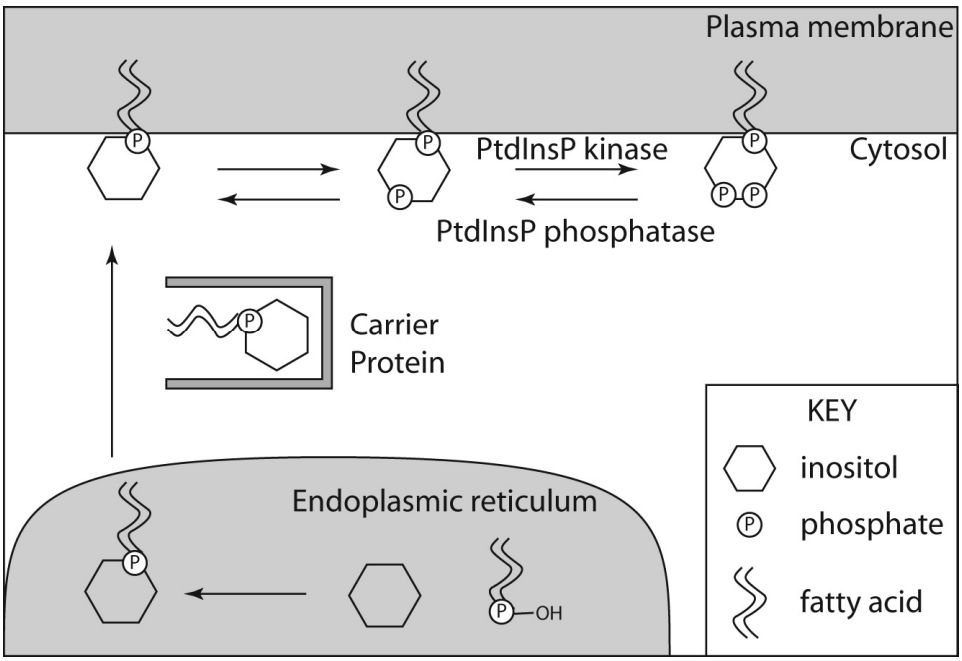


Figure 1.3: The generation of PtdIns(4,5)P_2 from PtdOH and *myo*-inositol.

In the 1980's the hormonally induced metabolism of PtdIns(4,5)P_2 was demonstrated to generate two secondary messengers; diacyl glycerol (DAG) and inositol 1,4,5-triphosphate [Ins(1,4,5)P_3].[17] Key to this process is phospholipase C (PLC) which is activated upon stimulation by an extracellular signal and hydrolyses PtdIns(4,5)P_2 into DAG and the Ins(1,4,5)P_3 head-group which diffuses into the cytosol, Figure 1.4.

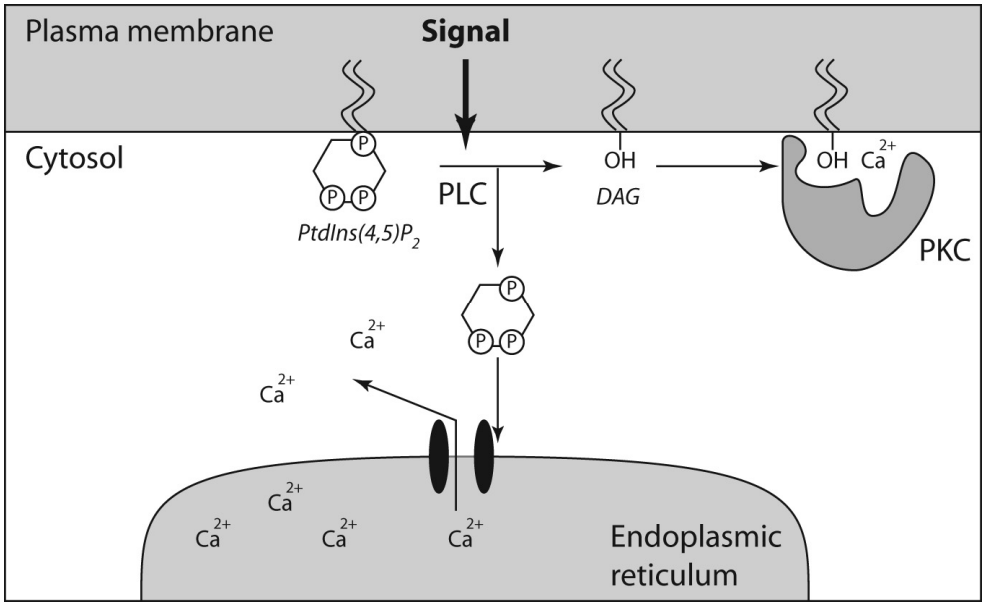


Figure 1.4: Upon arival of an external signal, PLC acts on PtdIns(4,5)P_2, to generate two second messengers; DAG and Ins(1,4,5)P_3

Nishizuka and co-workers[18] found that DAG reversibly activates protein kinase C (PKC) in the presence of acidic phospholipids and Ca^{2+}. Meanwhile, Berridge and co-workers[17] observed that an increase in the cellular concentration of Ins(1,4,5)P_3 preceded an increase in the

concentration of cytosolic Ca^{2+}, implicating this IP_3 as a secondary messenger effecting Ca^{2+} release. This second discovery provided the missing link between extracellular activation at the plasma membrane and the release of Ca^{2+} from internal stores. Binding of Ins(1,4,5)P_3 to receptors situated in the endoplasmic reticulum (ER), causing Ca^{2+} to flood from the ER Ca^{2+} store into the cytosol, has since been well characterised.[19] The revelation that PtdIns(4,5)P_2 was involved in vital cell functions, rather than just as a constituent part of the membrane, fueled an expanding research field.[19]

Rapidly generated second messengers also require efficient removal from the system in order to terminate their cellular effect. Dephosphorylation of Ins(1,4,5)P_3 deactivates this second messenger, generating first Ins(1,4)P_2 then InsP and finally inositol, which is recycled within the cell. It was later discovered that, following stimulation of cells, not only were these expected dephosphorylation products of Ins(1,4,5)P_3 present, but that other species including Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 emerged rapidly from Ins(1,4,5)P_3 metabolism.[13] Over 30 IP_n species have since been identified with phosphorylation observed on any of the six hydroxyls as well as identification of some pyrophosphates, termed IP_7 and IP_8.[20] In addition to the key role of Ins(1,4,5)P_3 in Ca^{2+} signalling, some other IP_ns have demonstrated roles in important functions including gene transcription, RNA editing, nuclear export and protein phosphorylation.[9] The sheer number of IP_ns, the diverse areas of cell biology in which they are implicated, the difficulty in understanding how their levels are regulated and poor availability of these species from nature has made them difficult targets to study.[13]

In the late 1980's, specific phosphorylation at the PtdIns 3-*O*H by phosphoinositide 3-kinase (PI3K) to generate PtdIns(3)P demonstrated that the PIP_ns were not purely precursors for cytosolic IP_ns.[21] All 7 PIP_ns resulting from reversible phosphorylation on any combination of the 3-, 4- and 5-*O*H were eventually reported,[23] Figure 1.5.

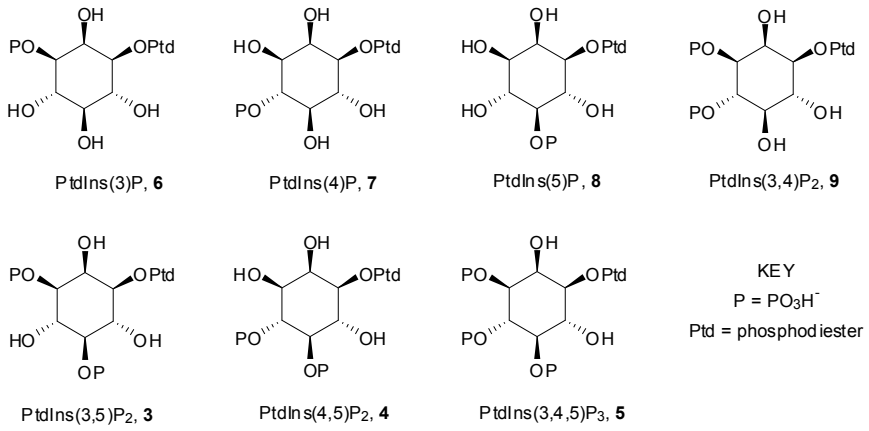


Figure 1.5: The 7 PIP_n which have been identified in biological systems

Contradicting the initial *in vitro* results, it was found that upon growth factor stimulation, class 1 PI3Ks act on PtdIns(4,5)P_2, phosphorylating the 3-*O*H to generate PtdIns(3,4,5)P_3,[22] Figure 1.6.

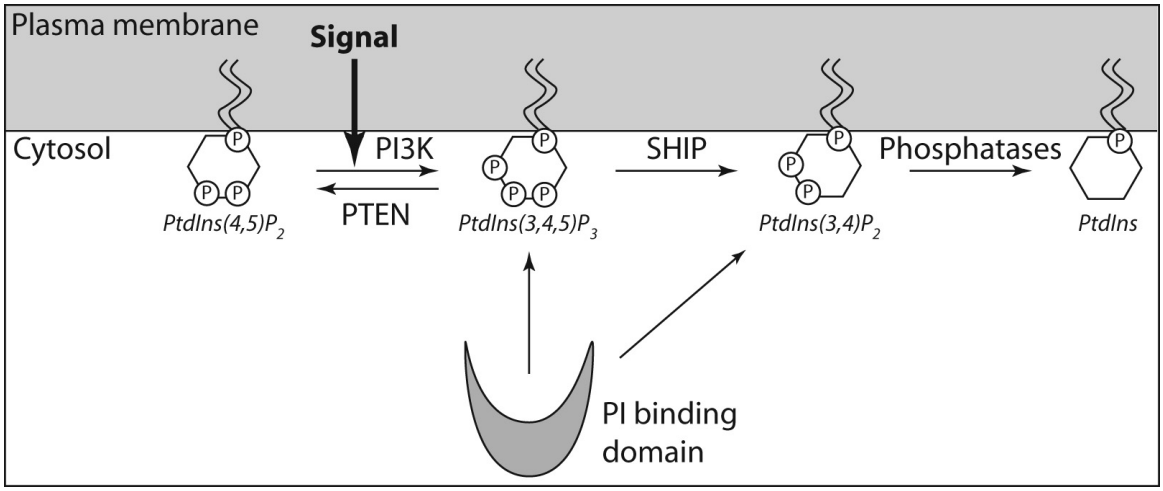


Figure 1.6: Upon stimulation by growth factors, PtdIns(4,5)P_2 is phosphorylated by PI3K to generate PtdIns(3,4,5)P_3. PtdIns(3,4,5)P_3 levels are tightly regulated by kinases and phosphatases.

Four of the PIP_n identified in eukaryotic cells are phosphorylated on the 3-*O*H; PtdIns(3)P, PtdIns(3,4)P_2, PtdIns(3,5)P_2 and PtdIns(3,4,5)P_3.[22] Resting mammalian cells contain significant levels of PtdIns(3)P, **6**, in internal membranes, but very low levels of the other 3-phosphorylated PIP_ns.[3] The levels of 3-phosphorylated phosphoinositides **3**, **5** and **9** are tightly regulated and typically rise many fold upon cellular stimulation.[14] For example, PtdIns(3,4,5)P_3 is present at ~0.1 % of the level of its precursor PtdIns(4,5)P_2 in resting cells. However, upon stimulation of tyrosine kinase receptors or some GPCRs, its concentration can increase by factors of between 2 and 100-fold.[24] This classical response to stimulation is central to the mechanism of action of the 3-phosphorylated species. Unlike PtdIns(4,5)P_2, the 3-phosphorylated PIP_ns are not substrates for mammalian PLC enzymes, but are modified by specific regulated kinases and phosphatases, as illustrated in Figure 1.6.[3]

These regulatory proteins act on the inositol ring, reversibly interconverting PIP_n species, resulting in short term alterations of their protein binding properties and functions. This ability to switch on or off a signal by the generation or removal of a given PIP_n make these species highly suited as effectors of membrane trafficking and cell signalling.[2] Following the generation of PtdIns(3,4,5)P_3, the inositol lipid phosphatases either counteract or diversify this signal.[3] 3-Phosphatases, including PTEN (phosphatase and tensin homolog deleted on chromosome 10) act on PtdIns(3,4,5)P_3, to counteract the effect of PI3K by dephosphorylating the 3-*O*, regenerating PtdIns(4,5)P_2.[25] The 4- and 5-phosphatases on the other hand, generate the PIP_2 species PtdIns(3,4)P_2 and PtdIns(4,5)P_2 which are also involved in signalling. For example, PtdIns(3,4,5)P_3 is the substrate for SHIP (SH2 domain containing inositol 5-phosphatase), which

dephosphorylates the 5-*O* to generate PtdIns(3,4)P_2.[22] The generation of further PIP_n species from PtdIns(3,4,5)P_3 places this PIP_3 at the centre of an increasingly complex signalling network.

The 3-phosphorylated PIP_n species are known to bind directly and selectively to specific modular domains expressed in a wide range of proteins and protein families.[2,26] A variety of structurally different PIP_n binding domains have been identified including the PH (pleckstrin homology), FYVE (deriving its name from the first four proteins in which it was identified; Fab1p, YOTB, Vac1 and EEA1), ENTH (epsin N-terminal homology) and PX (PhoX) domains.[1,26] The different domains display marked differences in selectivity between the different types of PIP_n, with some being more discriminating in their binding partners than others.[2] For example, all FYVE domains characterized to date bind PtdIns(3)P, whereas subsets of PH domains have been found to selectively bind PtdIns(3,4)P_2 and/or PtdIns(3,4,5)P_3 or PtdIns(3)P.[3] Binding to protein domains allows PIP_ns to regulate membrane-bound proteins and recruit cytosolic proteins to the membrane. The interaction of PIP_n with lipid binding domains transmits their signal, through activation, conformational change or phosphorylation, to the subsequent downstream stages of the signalling network. The 3-phosphoinositides, mainly through PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, have been shown to influence many different aspects of cell biology, including vesicle trafficking, cell growth and proliferation, DNA synthesis, regulation of apoptosis and cytoskeletal changes.[3,22,24] These widespread roles have led to an intense interest in signalling pathways downstream of PI3K as targets for therapeutics in disease control and prevention.[24] The availability of wortmannin[27] and LY294002,[28] both cell-permeable, low molecular weight inhibitors of the PI3K class of enzymes, has helped to elucidate some of the cellular functions these kinases and led to the implication of the PI3K pathway in abnormal cell growth.[3] PI3Kα is over-activated, due to mutation, in several significant colon, gastric and breast cancers.[6] Downstream from PI3K, PTEN has also been implicated in cancer due to its mutation in many late stage tumours.[6] Conversely, up-regulation of PTEN has been found to have anti-tumoural effects. The tumour suppressing function of PTEN is believed to be related to its 3-phosphatase activity which down-regulates activation of PI3K target proteins by reducing levels of PtdIns(3,4,5)P_3.[25] The results of these studies have further increased the desire to understand these mutations and their effects on disease.

These potent targets have proved difficult to study. It has not been possible to assign the exact combination of interactions downstream of PI3K or PTEN which lead to the pathological results. While a few PIP_n-protein domain interactions appear to be high affinity with dissociation constants in the nM range, over 80 % appear somewhat indescriminate in their PIP_n binding partners and have low binding affinities (in the μM range).[29] Binding tends to occur using a combination of specific head-group interactions, hydrophobic membrane penetration, electrostatic surface interactions and shape complementarity.[30] This combination of interactions with one or more additional binding sites within the membrane generates stable, high affinity interactions.[11,14]

For example, many small GTPases and GPCRs also bind the PIP_n-binding proteins and are located in the membrane, providing an additional binding site to generate a highly specific interaction.[14] Some PIP_n-binding protein interactions are known to induce a conformational change in the recruited protein which then permits the further modification or interaction of the protein at the membrane, for example by exposing particular residues, or altering localised membrane curvature.[2,31] The restriction of the PIP_ns to the membrane environment due to their fatty acyl tails allows them to be compartmentalised, determining their cellular location according to their specific function. This is believed to aid molecular recognition and demonstrates another way in which they impart specificity to their downstream targets.[3]

The pleckstrin homology (PH) domain is the best known PIP_n binding domain, found in over 500 diverse cell-regulatory proteins.[26,32] While having relatively low sequence homology, these domains are expressed as a highly conserved PH superfold, a seven-stranded β sandwich structure with a *C*-terminal α helix.[32] Most PH domains bind PIP_ns and therefore respond directly to changes in PIP_n levels as regulated by phosphoinositide-kinases and -phosphatases.[26] A subset of PH-domains have been shown to bind the 3-phosphorylated products of PI3Ks; PtdIns(3,4,5)P_3, PtdIns(3,4)P_2 and PtdIns(3,5)P_2.[32] Many PH-domain baring proteins require membrane association to function and studies of these PH domains fused to green flourescent protein have demonstrated the ability of PIP_n to recruit their binding proteins to the membrane, in a signal dependent manner.[32] A number of high resolution crystal structures of these domains have been reported, both in the absence of a ligand, and bound to an IP_n such as Ins(1,3,4,5)P_3 which mimics the PtdIns(3,4,5)P_3 headgroup,[31] Figure 1.7.

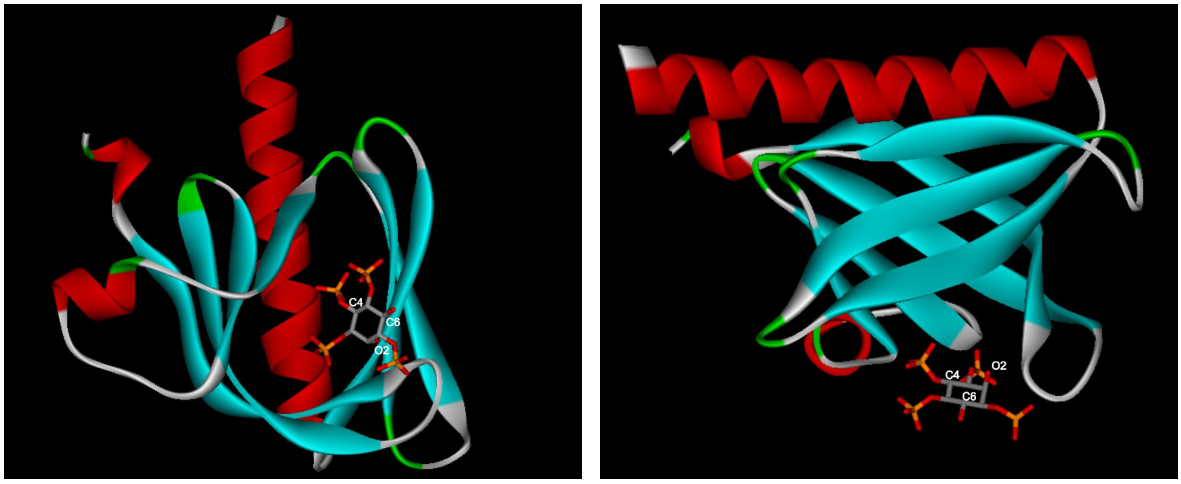


Figure 1.7: The PKBα-PH domain binding to inositol (1,3,4,5)-tetraphosphate.[33]

The high structural homology of the PH domains suggests that less well characterised domains may be probed successfully using techniques based on the known crystal structures. It is postulated that this will permit the elucidation of the function of many more PH-domain baring proteins once a suitable method has been developed.

More than one class of PIP_n receptor can be found in a single signalling protein,[26] so there is potential for cross-talk between lipid binding events, which has further complicated the elucidation of the exact functions of an individual protein. One particular example, which illustrates the need to disentangle these systems in order to understand them, is the activation of protein kinase B (PKB, also known as Akt). PKB has a well characterised *N*-terminal PH-domain, which specifically binds PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, and has emerged as a crucial regulator of widely divergent cellular processes including apoptosis, cell proliferation, differentiation and metabolism.[31] Over-activation of PKB has been reported to play a role in cancer initiation and progression. PKB was confirmed as a downstream effector of PI3K signalling by Franke *et al.*[34] who demonstrated that mutation of the PKB-PH domain prevents the response of PKB to PI3K. PKB is activated at the membrane and then appears to detach and translocate to both the cytosol and the nucleus. However the mechanism of activation is complex and not fully understood.[23] A second protein, phosphoinositide dependent kinase-1 (PDK-1) possesses a *C*-terminal PH-domain and binds to the same two PIP_n ligands as PKB.[23,35,36] PDK-1 is known to activate a number of different proteins, including PKB, by phosphorylating a Ser or Thr residue in their activation loop.[36] Therefore, upon generation of PtdIns(3,4,5)P_3 by PI3K, both proteins are translocated to the membrane, bringing them into close proximity. On binding to PtdIns(3,4,5)P_3, PKB has been shown to undergo a conformational change which allows phosphorylation by PDK-1.[31] Complete activation of PKB is thought to occur via a second phosphorylation by mTOR-rictor, before activated PKB diffuses into the cytosol to initiate its downstream effects. A better understanding of its activation and the ensuing cellular effects may point towards therapeutic possibilities.[37]

The unique versatility of the PIP_ns arising from their interconversion by kinases and phosphatases, their often transient nature, their spatial distribution and their interaction with a large range of effector molecules with specific PIP_n-binding domains, is both what permits the generation of such a vast range of downstream effects and what makes this system so hard to study.[24] While there is still plenty to learn about PKB activation and its downstream effects, there are many other PIP_n-protein interactions which have hardly been characterised. There is a pressing need to understand how these systems work: How do such similar molecules effect such different processes? How are individual signals converted into a cellular response? How does one and the same phosphoinositide, depending on the physiological context, trigger distinctly different events? This need is further compounded by the observation that specific signalling proteins which are downstream targets of PIP_n and IP_ns are overexpressed in disease states, such as cancer[6] and diabetes,[4] making these systems attractive targets for therapeutics. The elucidation of downstream signalling has been complicated by the huge range of different proteins which bind to phosphoinositides and cross-talk between signalling networks. Consequently, a method for observing the outcome of a single protein-ligand interaction is required.

When chosing targets to probe in this system, the key players PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 are attractive. PtdIns(4,5)P_2 participates in a vast array of events which occur at, or involve, the cell surface. It plays a major part in the transduction of extracellular signals, either via its metabolites, or by fluctuations in its own levels.[11] As the substrate for two classes of powerful receptor-regulated signal generating enzymes leading to two very different but important signalling cascades, PtdIns(4,5)P_2 is a focal point in PIP_n-dependent signalling.[6] There is also evidence that PtdIns(4,5)P_2 is a signalling molecule in its own right. Although its overall levels do not increase upon cell stimulation, it is likely that local increases in concentration can occur, for example at its sites of biosynthesis.[10] It is reported to recruit and bind to an increasing number of effector domains, including some ENTH and PH domains (such as the PH domain of PLCδ), to regulate actin polymerisation and anchorage, assembly of vesicular coats, endocytic vesicles and regulated secretion, among others.[24] The ability to selectively stimulate just one of these pathways in isolation would help to assign downstream observations to a particular role of this multifunctional signalling molecule.

The sheer number of proteins expressing binding domains which recognise PtdIns(3,4,5)P_3, the generation of PtdIns(3,4)P_2 by specific dephosphorylation and diverse range of downstream effects identified as a result of PtdIns(3,4,5)P_3-protein interactions make this a second valuable target. Separating out the responses to PIP_3 and where they lead in a cellular context would be invaluble to biochemical understanding as well as for therapeutic applications.

Some antagonists of these pathways have been identified: 3-Hydroxymethyl PIP_n analogues[38] and IP_5[39] have both been demonstrated to act on the PI3K pathway and therefore to suppress the signal from PKB which is thought to inhibit apoptosis in cancer cell growth.[38] However, in order to examine this system in detail, small molecule agonists are required that switch on the downstream effects, rather than off. In order to understand the complexity and sensitivity of the system, these observations will have to probe the cellular environment. The lack of this type of probe to date is a reflection of the challenge of generating such molecules. They need to be located at the membrane in order to effect the wild-type protein's translocation, and they need to specifically activate their target proteins as part of a concerted sequence of events.

1.3 Receptor Ligand Engineering

It is proposed here that using receptor-ligand engineering (RLE), one individual protein-ligand interaction can be studied *in vivo* in isolation from other signalling systems. This would be a powerful method for elucidating the downstream functions of specific interactions such as those described in the previous section. RLE is based on the modification of the specific binding arrangement between a protein binding site and its small molecule counterpart, to create a non-native pair.[40] By combining chemical modification of the ligand with complementary mutation of

its binding-protein, RLE uniquely pairs one chemical ligand with one protein target. This allows the investigation of their interaction in the context of the whole organism in a time and dose dependent manner.[41]

For a number of years, classical genetic techniques have allowed us to specifically affect the activity of a single protein within an organism and to observe the resulting effects. However, this is a slow technique as protein levels respond slowly to changes at the gene level and meaningful results are not guaranteed as the cell tends to compensate for disturbances in key signalling pathways by up-regulating other proteins.[41] In their genetic studies of mutant kinases, Bishop *et al*.[7] found that a signalling pathway of interest typically responded to a deleted kinase by increasing the production of closely related kinases. Therefore, during the 1-2 weeks they required for mutant genome expression, the effect of an individual deletion was usually masked. Furthermore, for PIP_n pathways, a single protein may possess more than one binding site, for instance for protein-protein docking, or act as a scaffold for other proteins to assemble around.[26] Consequently knock-out mutants that ablate an entire protein can display phenotypes unrelated to their lipid binding functions.

In response to the difficulties of using classical genetics to study such transient species, chemical genetics was developed.[42] By employing highly a specific chemical agonist or antagonist as a chemical switch, the activity of a target protein within an entire cell can be switched on or off.[43] The resulting effects can then be observed in a time and dose dependent manner, on addition of the small molecule, at diffusion controlled rates within the cell.[41] The term chemical genetics arose because the phenotype which is observed as a result of a small molecule ligand binding to its specific target may be thought of as functionally equivalent to mutating the protein. However, it is difficult to identify small molecules which interact very specifically with a desired target,[44] and the subsequent syntheses are often time consuming, expensive and may be of limited success.[42]

RLE combines the specificity of classical genetics with the versatility of chemical genetics.[40] A small molecule which interacts with a binding site of interest is modified using synthetic chemistry, so eliminating its ability to bind to the natural target. The small molecule may be either a natural ligand or one derived from screening synthetic libraries. Complementary genetic engineering of the protein binding-site then reconstitutes binding to the modified ligand.[42] By directing the protein modification to the binding site, only a small part of the overall structure is affected and any other binding sites within the protein should retain their natural functions. This is more precise and less intrusive than common deletion mutants.

Complementary modifications of small molecule-protein pairs have been carried out using two main methods:[40] Either a) by altering the shapes of the binding partners, known as steric complementation (or bump-hole) RLE; or b) by altering the polar or charged interactions in the binding site by manipulating H-bonds or ion pairs.

For steric complementation RLE a suitable bump, such as an alkyl or aryl group, is introduced on the small molecule ligand increasing the volume the ligand requires in the binding pocket and so preventing it from binding to the wild-type (WT) protein. This is complemented in a mutant receptor by the introduction of a void in the binding pocket where a large amino acid side-chain has been replaced by a small one, as illustrated in Figure 1.8.

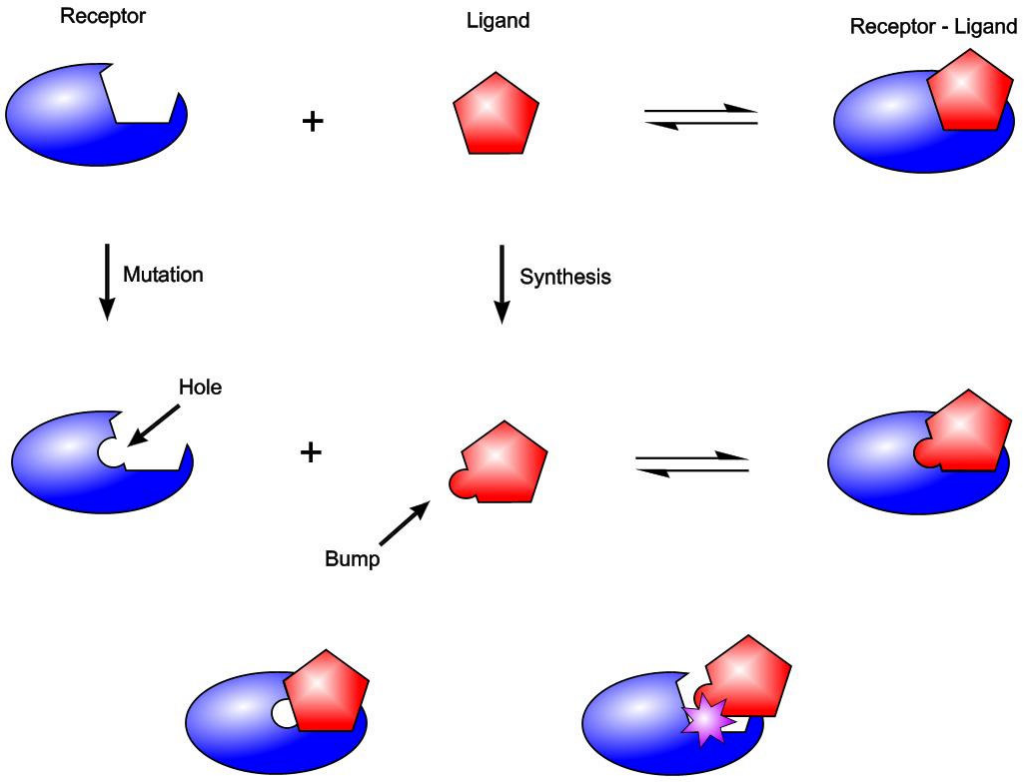


Figure 1.8: Schematic demonstrating the preparation of a specific receptor-ligand pair by steric complementation RLE

The specificity arises because the degeneracy of the natural system (where multiple proteins interact with the same small molecule ligand) is broken down to a one ligand, one protein interaction. It is a very controlled system because after expression of the unnatural protein in the cellular environment, the timescale and dose of introduction of the unnatural small ligand can be varied systematically. The compensatory up-regulation often observed in knock-out mutants is prevented as the native components can remain in the system. The natural ligand may still bind to the modified protein, however the modified ligand cannot interact with the WT protein.

Steric complementation RLE was first described by Schrieber and Belshaw[45] who re-engineered the complex formed by the immunosuppressive drug cyclosporin A and its intracellular soluble receptor, cyclophilin. This complex is a specific inhibitor of the protein phosphatase calcineurin and consequently has revealed some of the cellular functions of this protein.[45] It is thought that the interaction of the cyclosporin A-cyclophilin complex with calcineurin in cells outside the immune system is responsible for some of the undesirable side-effects associated with

this drug, such as hypertension, central nervous system toxicity and nephrotoxicity. Cyclophillins have been observed in nearly all organisms and tissues, so by generating a modified cyclosporin A-cyclophillin pair, and expressing the modified cyclophilin in one cell type, they were able to observe the tissue specific effects of calcineurin inhibition upon addition of modified cyclosporin A.[45]

Steric complementation RLE has also been employed to study signal transduction pathways (for a review see Bishop *et al.*[7]). Shokat *et al.*[46] combined point mutation of larger amino acids to glycine or alanine in the tyrosine kinase v-Src active site with complementary chemical modification of the natural ligand/co-substrate, ATP.[41] Their aim was to create a mutant kinase that would accept a modified ligand, A*TP, which was orthogonal to all other kinases. The mutant kinase required higher catalytic efficiency for A*TP than for natural substrate ATP, so that A*TP would preferentially phosphorylate the v-Src substrates. By studying the binding pocket of the native co-substrate ATP in similar kinases two amino acids were identified within a 5Å sphere of the ATP-N^6 docking point. Mutation of these amino acid residues to alanine created a pocket in the binding site which resulted in these mutants having lower ATP affinity than the WT protein. They then synthesised N^6-alkylated derivatives to create A*TP substrates which were orthogonal to the WT v-Src domain,[47] Figure 1.9.

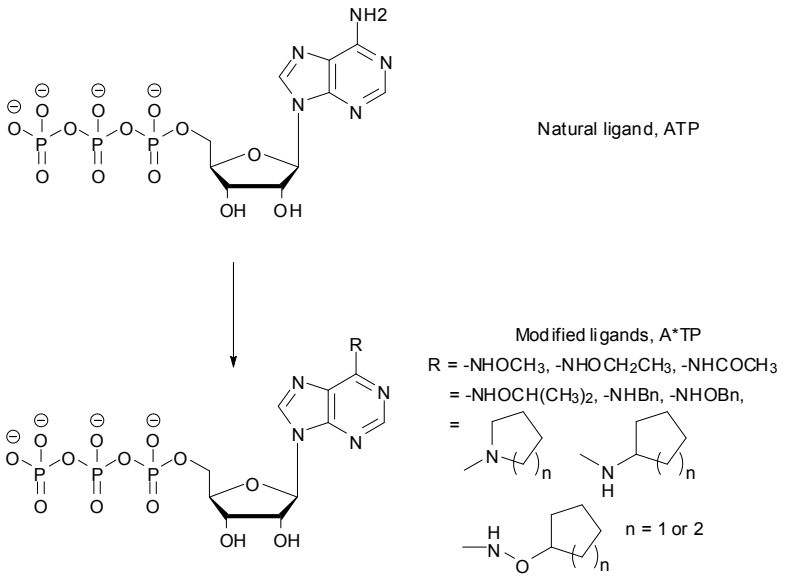


Figure 1.9: Chemical structures of ATP analogues (A*TPs) used to probe the nucleotide binding specificity of cellular protein tyrosine kinases.[47]

Screening these compounds against the modified kinase, they found that the N^6-(benzyl) ligand (R= -NHBn, Figure 1.9) bound selectively to the mutant protein even at levels similar to the cellular concentrations of native ATP. By incorporating a radio-active ^{32}P into the terminal γ-P of the triphosphate moiety of N^6-(benzyl)-A*TP they were able to label and identify the downstream substrates phosphorylated by v-Src.[47] This work demonstrated that engineering of an active site

was possible without affecting kinase activity. Using this approach, they also designed several drug-like nanomolar inhibitors of engineered kinases by applying steric complementation RLE to to increase the specificity of non-specific inhibitors.[41] These modified inhibitors do not significantly inhibit the WT kinases allowing them to be targetted to a kinase of interest.[41]

Lin *et al.*[48] used steric complementation RLE to study protein arginine methyltransferases. This family of enzymes catalyse the transfer of methyl groups from S-adenosylmethionine (SAM) to arginine side chains in their target proteins, thereby regulating transcription, RNA processing and receptor-mediated signalling.[48] Substitution at the SAM-N^6 (similar to ATP-N^6) and complementary mutation of RMT1 (arginine methyl transferase 1), created a specific orthogonal protein-ligand pair. By incorporating a ^{14}C radio-label they were able to identify the specific targets of methyl transfer catalysed by this individual enzyme *in vivo*.[48] Furthermore, similar modification of S-adenosylhomocysteine (SAH), which remains after methyl transfer from SAM, combined with a complementary RMT1 mutation generated inhibitors which could be used to selectively prevent methyl transfer by this protein *in vivo*.[48]

In these examples of steric complementation RLE, the synthetic modification of the ligand and protein mutation have been successfully applied to the entire class of ATP-binding and methyl transferase proteins, allowing each individual interaction to be probed in turn. Such a general method would be ideal for the step-wise investigation of PIP_n signalling networks.

Small molecule-protein binding is not only shape specific but almost always involves complementary charge interactions.[40] Manipulation of these key polar binding interactions (such as the number and direction of hydrogen bonds or the strength and direction of salt-bridges) offers another avenue for modification of the natural interaction to generate specific binding pairs. This second approach reduces the association between the modified protein and the natural ligand which is often observed in steric complementation studies,[7] making these two approaches somewhat complementary.

One of the first examples of manipulating polar interactions was Hwang and Miller's[49] examination the role of the protein elongation factor-Tu (EF-Tu) and its natural ligand guanine triphosphate (GTP) in protein biosynthesis. Binding studies revealed that the guanosine base of GTP had several hydrogen bonds to amino acids in the EF-Tu binding pocket. Disruption of one key hydrogen bond between aspartic acid-138 (Asp-138) and GTP by replacing the amino acid with asparagine (Asn) changed an H-bond acceptor into an H-bond donor. Replacing GTP by xanthine triphosphate (XTP) then provided the new donor with a complementary H-bond acceptor. This interaction could then be studied without interference from other signalling events. The H-bond reversal successfully permitted the subsequent investigation of other related GTP binding proteins.[49]

Exchange of H-bonds in RLE requires the careful design of specific mutants as these charged interactions are directional, and the reversal of one H-bond can often cause disruption to wider H-bond networks. The neutralisation, reversal or exchange of ion pairs within the binding site has also been used to generate specific receptor-ligand pairs. Unlike H-bonds, these charged interactions are non directional and are therefore easier to manipulate. However, for the highly negatively charged PIP_n lipid headgroup, the introduction of a positive charge, or neutralisation of an ion pair may significantly reduce the binding affinity of the modified ligand. Ion pair exchange, which could be used to introduce a negatively charged protrusion onto the ligand, would be a viable method for RLE of PIP_n-protein interactions. However, the generation of polar exchange RLE pairs requires an intricate knowledge of the binding site, such as a known crystal structure. While this is possible for some PIP_n-protein interactions with published crystal structures, such as the PKB-PH domain bound to IP_4, it is not possible for the large number of PIP_n binding domains which have not been co-crystallised with an inositol phosphate ligand.

1.4 Applying RLE to PIP_ns

With the ultimate aim of being able to characterise the downstream consequences of any given PIP_n-binding protein interaction, it is intended to prepare unique protein-ligand binding pairs using steric complementation RLE, as illustrated in Figure 1.10.

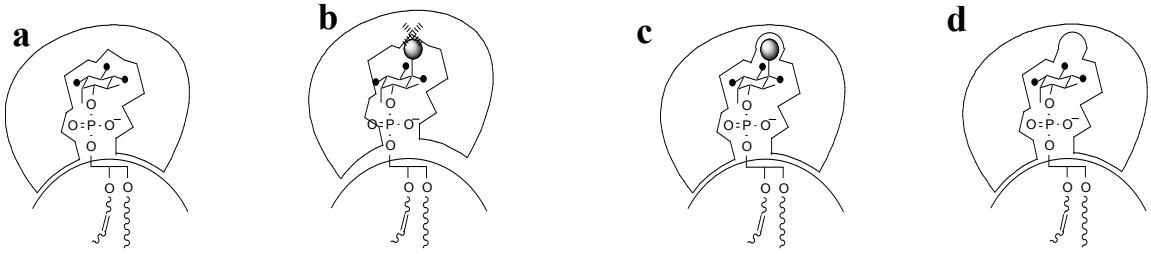


Figure 1.10: Applying the bump-hole approach to PIP_n-protein binding (a) natural protein-lipid binding; b) addition of a steric bump to the lipid; c) accommodation of the bump in the protein binding site; d) the natural lipid may still bind the mutant protein.

It is hoped that once suitable inositol head group-lipid binding domain pairs have been established, the full length mutant lipid binding protein can be expressed *in vivo* and the modified lipid added, to study a whole range of PIP_n-protein interactions. In the natural system, a membrane bound inositol lipid binds to many related protein binding domains which results in a multitude of downstream signalling events (e.g. multiple PH-domains binding to PIP_3), Figure 1.10a. Since the lipid can bind to many related proteins, treating a cell with the natural lipid can cause a multitude of different downstream effects, making interpretation of the experiment and understanding of the system difficult. A small additional substituent (the 'bump') is incorporated onto the lipid, which prevents it from binding to its WT target proteins, as there is a steric clash in the binding pocket

with the bump, Figure 1.10b. The modified lipid is then said to be orthogonal to the WT protein. Next, a single signalling protein of interest is engineered to accept the orthogonal ligand, by replacing one or two residues in the binding pocket by amino acids with smaller side chains. This creates an artificial void, which can accommodate the steric addition to the lipid, generating a unique binding pair. The mutation must not interfere with any other aspect of protein function, Figure 1.10c. The mutated protein is engineered into a cell line to investigate the effect on the signalling system when the modified ligand is added. The original shape and size of the binding pocket has not been decreased so even if the WT protein is deleted the mutated protein can still accept the natural ligand, Figure 1.10d. Hence there can be no re-routing of the signalling system to avoid the mutant protein.

Using the known crystal structures[31,32] of IP_n lipid head-groups bound to lipid binding domains, it is perhaps possible to propose suitable mutations and chemical manipulations for a steric complementation pair. For example, in the crystal structure of the PKBα-PH domain bound to Ins(1,3,4,5)P_4, amino acids in the binding site which are electrostatically involved in phosphate recognition must be retained to maintain binding affinity, Figure 1.11a. On the other hand, amino acids which are involved in shaping the binding pocket offer possibilities to introduce a void, Figure 1.11b.

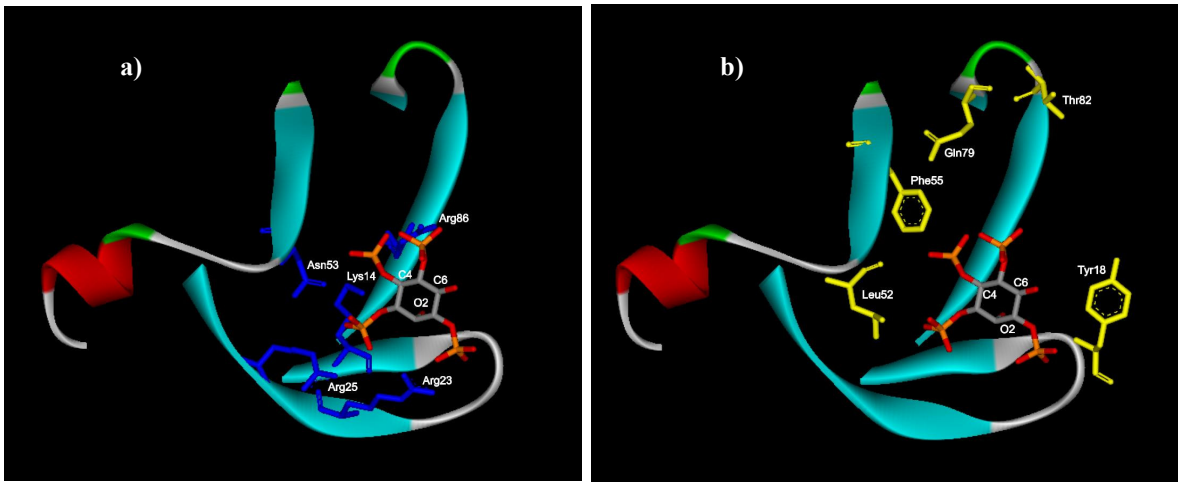


Figure 1.11: PKBα-PH domain binding to Ins(1,3,4,5)P_4 a) Amino acids with electrostatic binding interactions; b) Amino acids with purely steric input at the binding site.[33]

By replacing a larger amino acid (e.g. phenylalanine) with a smaller amino acid (such as alanine) we create the required void to accommodate a steric protrusion on the ligand. Glycine, the smallest amino acid, could be used, but because it has no alkyl side chain it is more likely to affect the protein backbone flexibility which may undesirably distort the native fold of the protein.

Suitable chemical modifications to the PIP_n ligand can be proposed by examination of the inositol ring orientation in the known co-crystal structures with binding proteins. Generally, the 3-, 4- and 5-positions of the ring will lie deepest in the binding pocket, as these are furthest from the 1-

O-lipid tail which is anchored in the membrane. The PH-domain structures suggest that the head group binds with one face parallel to the binding pocket of the protein, although the orientation of the inositol ring can be reversed depending on the structure of the individual binding pocket,[3] Figure 1.12.

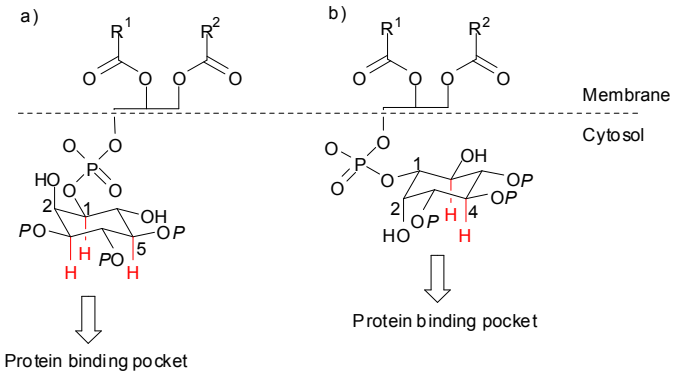


Figure 1.12: The orientation of PIP_ns in the binding pocket

The phosphate groups on the PIP_n are responsible for the electrostatic interactions upon binding, so altering these moieties risks reducing the binding affinity. However, the C-H protons around the inositol ring are not involved in electrostatic interactions. Replacing one of these protons with an alkyl group offers an opportunity for introduction of a steric protrusion. In the natural conformation, five of these steric protrusions would be axial (1, 3, 4, 5 and 6) and therefore could point directly at the surface of the protein during binding, Figure 1.12. The 2-position contains an axial hydroxyl with the inositol proton occupying an equatorial site, and lies adjacent to the 1-*O*-lipid tail. Consequently a bump here may not project so directly into the protein-ligand interface, making this a less desirable place for introducing a steric protrusion.

Following the generation of mutated proteins and chemically modified ligands, a method for assessing the binding affinity between a given ligand and protein is required so that a successful RLE protein ligand pair can be identified. Classical screening of mutated ligands and proteins to find a binding pair has been carried out using affinity chromatography or by immobilising the protein or ligand on a solid support,[50] which allows large numbers of ligands and proteins to be screened at one time. Rao *et al.*[51] used biotinylated PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 bound to avadin coated beads to identify proteins which bound 3-phosphorylated PIP_ns. Using this technique they isolated three proteins from mouse spleen. Similarly, Hammonds-Odie *et al.*[52] identifed Ins(1,3,4,5)P_4 binding protein Centaurin-α using affinity resin and photoaffinity labeled phospholipid analogues of IP_4. That these techniques have only served to identify a small fraction of the proteins known to bind PIP_n and IP_n species, is testamony to the complex nature of the PIP_n-protein binding which is often relatively low affinity,[29] with a concerted mechanism of binding to other membrane features. The inability of such screens to recreate these high affinity concerted interactions means it is likely that valid mutant protein-bumpy ligand pairs may be missed.

Therefore it is planned that screening to identify unique lipid-protein binding pairs will be carried out *in silico*.[†] This requires the docking of candidate ligand libraries into potential lipid binding protein targets to identify possible mutants which may accommodate the modified ligand. The interactions of such highly charged species are difficult to model, and therefore to ensure that meaningful results are obtained from *in silico* studies, it is intended that the model will be validated using real binding data. By generating ion-pair exchange RLE data using mutant proteins already available in the group and modified IP_4 ligands designed to fit the well known PKB-PH domain, it should be possible to collect real binding values for given protein-ligand interactions. Isothermal titration calorimetry (ITC)[53] is being used to measure the change in enthalpy which occurs upon introduction of a modified IP_4 to a mutant PKB. These values will be compared with the predicted values from the *in silico* model to ensure that it accurately recreates the binding event.

Once a mutant protein-bumpy phosphoinositide pair has been predicted by the *in silico* model, ITC can be used to confirm and quantify the interaction between the designed ligand and mutant protein, to ultimately generate a specific binding pair which can be used within the context of the whole cell. The PIP_n ligand must then be synthesised in full in order to ensure it's location at the cell membrane for whole cell studies.

Following the identification of a specific protein-ligand pair, the protein must be engineered into the cell. It is then hoped that the lipid ligand can be delivered to the cell to observe the effects of this one interaction in a time and dose-dependent manner. This in itself is a complex task which will require optimising in order to introduce the modified PIP_n to the inner leaflet of the plasma membrane. By comparing the cell content before and after introduction of the bumpy ligand using proteomic strategies, it is anticipated that the specific downstream effects from one signalling event may be observed.

Underpinning this strategy to elucidate PIP_n signalling, is the requirement to prepare PIP_n ligand analogues with a steric protrusion, using chemical synthesis. The preparation of such analogues has not been reported in the literature to date, and would provide new tools for the study of phosphoinositide systems in a much wider context than the RLE system for which they are designed. The preparation of these analogues represents a considerable challenge in itself, and is the focus of the work within this thesis.

[†] This project is currently being undertaken by an MRes/PhD student, in collaboration with Ian Gould at Imperial College.

Chapter 2: Chemistry Introduction

This chapter reviews the tools available for the synthesis of IP_n and PIP_ns and will explain why the 4-*C*-alkyl IP_3 and IP_4s were chosen as initial synthetic targets for this work. By reviewing the available literature, a synthetic strategy was designed, which is introduced here in terms of the four main requirements for chemical synthesis; protection of a suitably chosen starting material, followed by introduction of a steric protrusion suitable for RLE, subsequent rearrangement of protecting groups and finally the phosphorylation and deprotection to generate the target molecules.

The preparation of IP_n and PIP_ns has proved challenging due to the requirement for regiospecific protection of inositol, phosphorylation of a polyol, removal of phosphate protecting groups while avoiding migration, and purification of the final water soluble poly-anions.[54] Combined with the limited availability of these important signalling molecules from Nature, this has led to a wealth of synthetic routes.[15,54,55] Initial synthetic targets were the natural products themselves, although the identification of these molecules in pathways whose perturbation leads to disease states rapidly led to the preparation of analogues for biological studies.[54] In addition to the inherent challenges of IP_n and PIP_n preparation, the proposed synthesis of *C*-alkyl PIP_ns must address the stereo- and regiospecific introduction of an alkyl group, replacing an inositol ring C-H.

In order to address this new challenge an expedient route to a robustly protected inositol with a single free hydroxyl was sought. This intermediate must tolerate, and preferably direct, the introduction of a *C*-alkyl group after oxidation. Evaluation of protecting group strategies suggested that the 4-*C* could be easily isolated using an orthoester, and that this protecting group could be later manipulated to expose the hydroxyls of IP_4 analogues for phosphorylation. It was envisaged that 4-*C*-alkyl introduction would proceed via inos-4-ose, **11**, Figure 2.1.

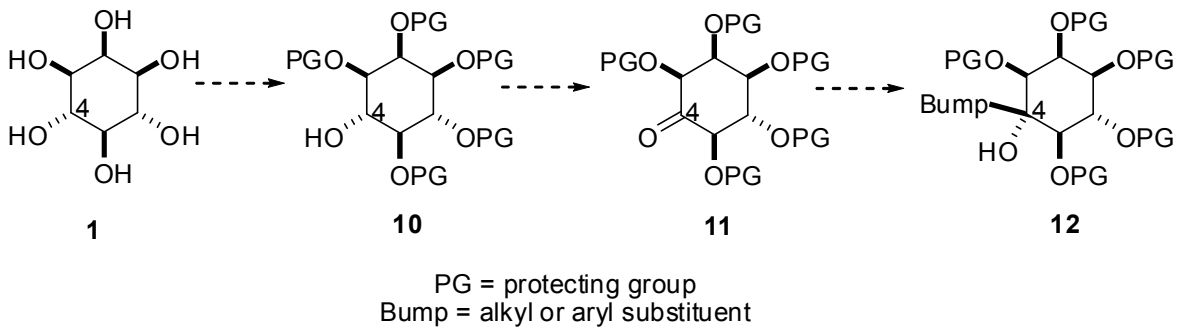
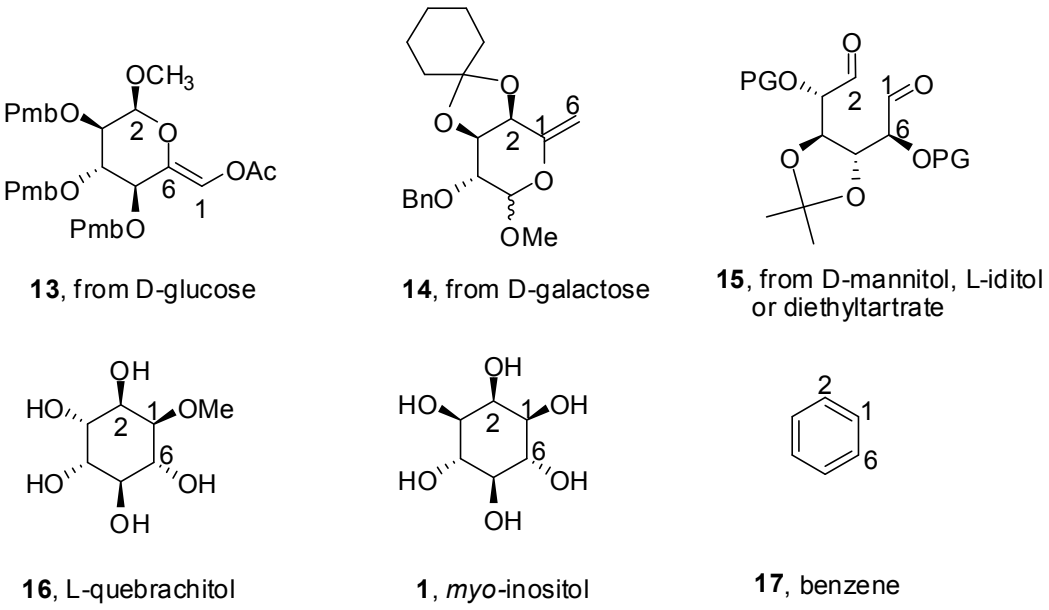


Figure 2.1: Introduction of an alkyl group involves oxidation of the 4-*O*H

Stereocontrolled addition of an alkyl equivalent to the inosose would regenerate the 4-*O*H, **12**. These derivatives could then be taken on to investigate protecting group manipulations to generate a selectively protected triol or tetrol, followed by phosphorylation and deprotection. In order to demonstrate that *C*-alkyl analogues of PIP_ns were feasible targets, it was essential that all three of these subsequent transformations were possible in the presence of the unnatural tertiary centre. Therefore it was decided to prepare the IP_n head group, rather than the full PIP_n lipid analogue which requires differentiation between the hydroxyls and construction of the phosphatidate moiety. This matter takes up the entirety of this thesis. Since it is the inositol phosphate head group which forms the protein binding site, the IP_n analogues should not only prove the validity of the chemical transformations, but also provide an adequate model for initial binding studies. Once the chemistry is established and specific targets have been identified for RLE, the synthesis of the corresponding PIP_n derivatives can be undertaken.

2.1 Starting Materials

The reported starting points for the synthesis of inositol phosphates fall into two classes, either a) from materials with built-in asymmetry, originating from the chiral-pool, or b) from *meso* *myo*-inositol or benzene which require a later chemical or enzymatic resolution of enantiomers, Figure 2.2.



13, from D-glucose

14, from D-galactose

15, from D-mannitol, L-iditol or diethyltartrate

16, L-quebrachitol

1, *myo*-inositol

17, benzene

Figure 2.2: Starting materials for inositol phosphate synthesis, numbered according to the position of carbons in the subsequent *myo*-inositol framework

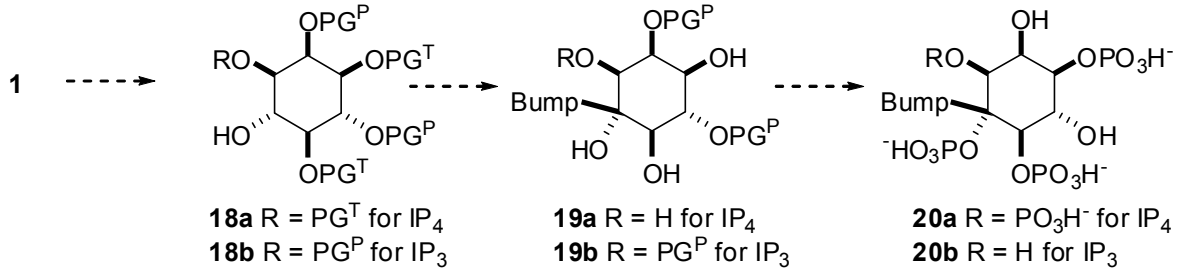
Simple sugars are attractive starting materials for the preparation of chiral inositols as they are available enantiomerically pure at relatively low cost. Protection of hydroxyl functionalities is used to generate a key intermediate such as **13**, **14**, or **15**, which will undergo a rearrangment or coupling, transmitting chirality to the newly formed inositol framework. For example, the Ferrier

rearrangement[56] of **13** derived from D-glucose was used to prepare Ins(1,4,5)P_3[57] and 1-*O*-functionalised IP_4 affinity probes.[58] Using a similar strategy, chiral 6- and 3,6-deoxy-D-*myo*-inositol precursors were prepared from D-galactose derivative **14**.[59] D-Mannitol,[60] L-iditol[61] and diethyltartrate[62] have been used to prepare dialdehydes such as **15** which undergo samarium iodide mediated stereoselective reductive couplings to form enantiomerically pure *myo*-inositol frameworks. Chiral materials other than sugars have also been employed as starting materials: Naturally occurring L-quebrachitol [1-L-(-)-2-*O*-methyl-*chiro*-inositol] **16** is commercially available as a side product of the rubber industry,[63] although it is more expensive than alternative materials (£48.30 for 250 mg from Sigma-Aldrich). Protection of some or all of the free hydroxyls, inversion of the 3-*O*H and demethylation of the 1-*O* (numbering for the *myo*-inositol product) transmits the chirality of the starting material to a *myo*-inositol derivative.[63] One disadvantage of such syntheses, is the number of manipulations required prior to the generation of the inositol framework, which must often be followed by rearrangement of protecting groups to generate a building block suitable for phosphorylation and deprotection to prepare the desired IP_n or PIP_n.

In each case, these chiral syntheses only generate one of the two possible enantiomers of the final IP_n or PIP_n and it was for this reason that the *meso*-cyclohexane hexitol *myo*-inositol **1** was the starting material of choice to prepare *C*-alkyl IP_ns. The need to carry out a resolution of the racemic material during synthesis starting from **1** was considered an advantage for the RLE project, as both the natural and unnatural enantiomers could ultimately be prepared. This provides an ideal control for biophysical studies as the unnatural enantiomer can be used to assess the effects of adding a non-interacting PIP_n lipid into the cell. The pre-installed *myo*-stereochemistry and cyclohexane ring make *myo*-inositol an attractive choice for rapidly obtaining large quantities of material with a suitable protecting group strategy to test the introduction of *C*-alkyl groups. Furthermore, it is available at reasonable cost from chemical suppliers (£11.20 for 50 g from Sigma-Aldrich). For these reasons, *myo*-inositol has been a popular starting material in the preparation of IP_ns, and the protection and deprotection of the six secondary hydroxyls to permit selective functionalisation has been extensively reported.[64-66] Benzene **17** has also been used as a starting material;[67] like *myo*-inositol, it is cheap and readily available. The absence of any predefined stereochemistry or substituents provides a blank canvas for functionalisation. Syntheses are racemic, starting with oxidation of benzene to introduce hydroxyls. Related chiral syntheses have started from enzymatic dihydroxylation of chlorobenzene to selectively generate one enantiomer for further elaboration. However, the extra steps required to selectively introduce the hydroxyls, often involving temporary protecting groups, mean that the route to a fully protected intermediate from benzene is not as expedient as with *myo*-inositol.

2.2 The introduction of protecting groups to isolate the 4-*C*

With *myo*-inositol as a starting point for inositol phosphate synthesis it is necessary to manipulate, and differentiate between, the six similar secondary hydroxyls. There are two key intermediates which the protecting group strategy must facilitate access to: The first is the isolation of a hydroxyl for manipulation to generate **18a** and **b**. However, this has to incorporate the second requirement, which is that in the later stages of the synthesis, hydroxyls required to undergo phosphorylation must be unprotected (the 1,4,5-triol for IP_3 and the 1,3,4,5-tetrol for IP_4), while hydroxyls of the target are protected (the 2,3,6-*O* for IP_3 and the 2,6-*O* for IP_4), **19**, as illustrated in Figure 2.3



Key: PG^T = Temporary protecting group, PG^P = Permanent protecting group.

Figure 2.3: Protecting group strategy during synthesis of a 4-*C*-alkyl IP_4 analogue

This strategy requires two types of protecting group; permanent protecting groups which remain in position until the final deprotection of the target molecule, and temporary protecting groups which can be selectively removed prior to phosphorylation. After introduction of the *C*-alkyl substituent, it is essential that the temporary protecting groups be selectively removed in the presence of the permanent protecting groups. Manipulation of the free hydroxyl of fully protected **18a** and **b** involves oxidation of a C-H bond to a C-C bond, so both the temporary and permanent protecting groups must withstand the conditions required to effect this transformation. Furthermore, *C*-alkyl introduction must be stereospecific so any directing influence which aids stereocontrol, for example using steric bulk of protecting groups, would be beneficial.

Many strategies for the isolation of hydroxyl groups have been developed.[64,65] If starting a synthesis from *myo*-inositol, initial protection of two, three or four hydroxyls has typically been carried out using either ketals or orthoesters, Figure 2.4.

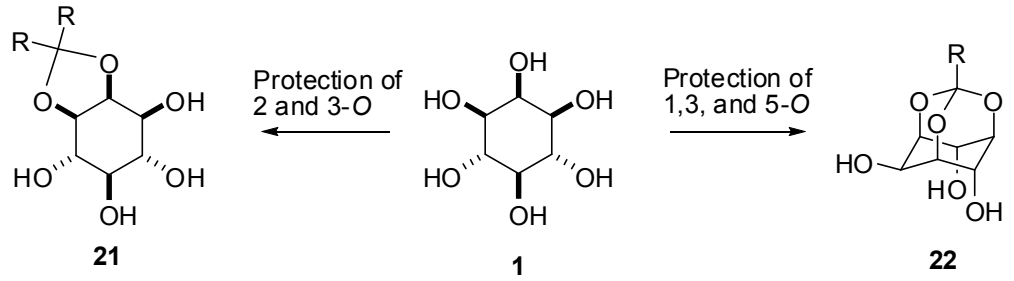


Figure 2.4: Orthoester and ketal groups as initial protecting groups for *myo*-inositol.

Both methods have been used to prepare protected inositols in high yields on large scales, making them ideal starting points for synthesis.[64,65] *myo*-Inositol mono- and bis-acetals have been obtained in good yield, protecting up to four hydroxyls simultaneously.[64] Ketalisation of *myo*-inositol under forcing conditions results in the formation of a mixture of bis-ketal isomers, which are separable by crystallisation. Orthoesters of *myo*-inositol have become prominent in the literature in recent years, due to their easy isolation in multi-gram quantities[68] and the simultaneous protection of the 1-, 3-, and 5-*O* forming a rigid adamantane-like cage.[64] *myo*-Inositol orthoesters are prepared by the reaction of *myo*-inositol with the triethyl or -methyl orthoester of the desired carboxylic acid under acid catalysis.[68] The reagent exchanges ester bonds with inositol hydroxyls, equilibrating between a series of intermediate structures, until the most stable, tricyclic species forms. For *myo*-inositol, with five equatorial and one axial hydroxyl, there is only one such possibility, the 1,3,5-*O*-triester, Figure 2.5a. By contrast, the similar transesterification of *scyllo*-inositol, **23**, which has all six hydroxyls equatorial forms the diorthoester **24**,[69] Figure 2.5b.

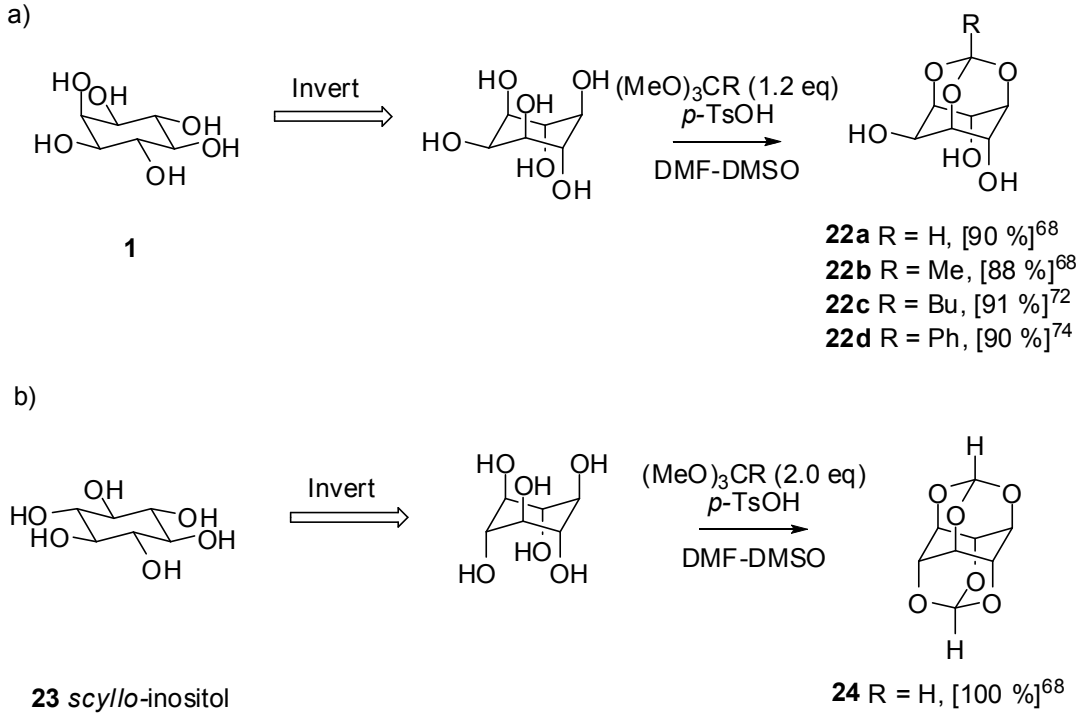


Figure 2.5: a) The preparation of orthoesters from *myo*-inositol, b) The preparation of *bis*-orthoester from *scyllo*-inositol

Lee and Kishi[70] first fully characterised the orthoformate **22a**, Figure 2.5a, in 1985 and it has been the most thoroughly investigated of the *myo*-inositol orthoesters. Recently, more varied orthoesters have been employed in IP_n synthesis, including the orthoacetate,[68,71] orthopentanoate[72] and orthobenzoate.[73,74] In particular the orthobenzoate, **22d**, is reported to be easily recovered by recrystallisation from ethyl acetate.[73]

When considering which initial protecting group to employ for the preparation of *C*-alkyl inositol phosphates, the simultaneous protection of the 1-, 3- and 5-*O* offered by an orthoester is attractive as either two (for IP_3) or all three (for IP_4) of these hydroxyls will be phosphorylated at a later stage. Their simple, high yielding preparation should permit large quantities of protected material to be carried through to later stages of the synthesis. It was also anticipated that the orthoester would be more stable than the corresponding ketal during oxidation, essential for the proposed strategy for introducing a *C*-alkyl group via oxidation of the 4-*O*H to the inosose, Figure 2.1. On oxidation of an inositol hydroxyl, isomerisation of the resulting inosose to the enolate must be avoided in order to prevent elimination of the ring substituents. The steric constraint of the orthoester should prevent tautomerisation, as the adjacent bridgehead carbons prevent the enolate double bond from achieving the required co-planarity. Attempted elaboration of conformationally unrestricted inososes under basic conditions has been reported to result in elimination to unsaturated products.[75]

After introduction of the orthoester, protecting two of the remaining three hydroxyls will isolate a single hydroxyl for manipulation. Of the 2-, 4- and 6-*C*, the 4- or 6-position were identified as suitable sites for *C*-alkyl substituents (see Chapter 1.4). In the protecting group strategy proposed in Figure 2.3, the 2- and 6-*O*H require permanent protecting groups for both IP_3 and IP_4 products. Therefore it was anticipated that introduction of a 4-*C*-alkyl group by manipulation of the 4-*O*H would require the least number of additional protecting group manipulations.

The orthoester locks the conformation of the cyclohexane ring, so that the remaining equatorial 2-*O*H occupies a very different environment to the axial 4- and 6-*O*H. As a result, the regioselective protection of the 2-, 4- and 6-*O* with alkyl, acyl, sulfonyl and silyl protecting groups is extensively described in the literature and differentiation of any individual hydroxyl from the remaining two has been reported.[64-66] The 4- and 6-*O*H form a strong intramolecular hydrogen bond, the result of which is that one of these more hindered hydroxyls may be selectively deprotonated before the 2-*O*H with a strong base, Figure 2.6.

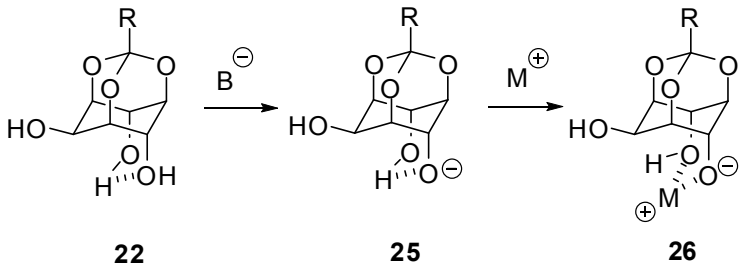


Figure 2.6: Stabilisation of 4(6)-*O*-anion and chelation of metal cation

The resulting anion **25**, is stabilised by intramolecular hydrogen bonding with the remaining axial hydroxyl group. When the base is a metal hydride, the metal ion may be chelated in the di-axial 4,6-*O* pocket, **26**, as initially postulated by Billington *et al.*,[76] resulting in selective 4(6)-*O* protection with a wide variety of groups. This selectivity has been exploited to form many *myo*-inositol orthoester alkyl derivatives at the more hindered 4(6)-*O* with 1 equivalent (eq.) of sodium hydride, followed by addition of an alkyl halide, Figure 2.7, **29**. However, alkylation with 2 eq. sodium hydride results in a mixture of 2,4- and 4,6-*O*-dialkylated species **28** and **30**, due to the opposed effects of increased steric hindrance at the remaining axial hydroxyl and chelation of the metal in the diaxial pocket.[66] Deveraj *et al.*[66] report that 4,6-*O*-dialkyl derivatives **30** can be selectively generated using BuLi to effect the second deprotonation due to improved chelation of the small Li-cation in the tight 4,6-*O*-pocket, Figure 2.7.

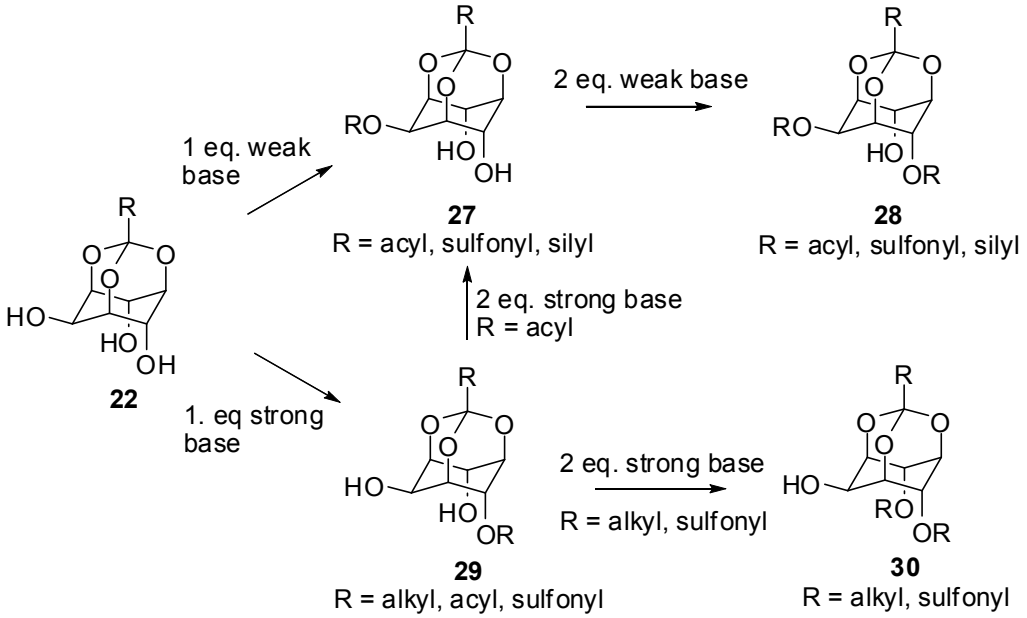


Figure 2.7: Selective protection of the 2-, 4- and 6-*O*

The initial regioselectivity of acylation or sulfonation can be influenced by the choice of organic base; use of a weak base such as pyridine preferentially protects the 2-*O* whereas a stronger base such as triethylamine can weakly deprotonate therefore protect the 4(6)-*O*.[77] Thus, in the presence of pyridine, 2,4(6)-*O*-disulfonyl or -diacyl esters are selectively generated by a second reaction at the 4(6)-*O*.[78,79] For the sulfonates, this selectivity can be reversed by use of excess sodium hydride to prepare the 4,6-*O*-disulfonates[78] with increased selectivity compared to the dialkyl species, possibly due to the enhanced chelation of the sodium metal with the initial 4(6)-*O*-sulfonate.[78] However for the acyl esters, while 1 eq. sodium hydride deprotonates and acylates the 4-*O*, addition of 2 eq. sodium hydride in DMF surprisingly generates the 2-*O*-acyl ester. Sureshan and Shashidhar[80] report that addition of sodium hydride to a mixture of 4-*O*-acetyl orthoformate and 4-*O*-benzoyl orthoacetate in DMF yielded the 2-*O*-acetyl orthoformate and the 2-*O*-benzoyl orthoacetate resulting from acyl migration. No cross-products of intermolecular acyl transfer were observed.[80] Their results suggest that the observed acyl transfer occurs intramolecularly, although direct acyl transfer from the 4-*O* to the 2-*O* is clearly geometrically impossible. The reaction is dependent on the presence of sodium hydride and DMF; when carried out in THF, 4-*O*-benzoyl migration was observed at a much slower rate, in a non-selective manner, generating a mixture of all possible mono-, di- and tribenzoyl products, suggesting that the solvent may act as a shuttle. Selective deprotonation with 1 eq. sodium hydride can be extended to bulky groups such as *tert*-butyldimethylsilyl (Tbdms) to prepare 4-*O*-Tbdms derivatives, whereas under mildly basic conditions, Tbdms reacts preferentially with the less hindered 2-*O*, followed by the 4-*O*, to generate the asymmetric 2,4-*O*-$Tbdms_2$ derivative,[81] Figure 2.7.

To isolate the 4-*C*, protecting groups must be introduced on the 2- and 6-*O* of the *myo*-inositol orthoester. As demonstrated above, this is possible in one step using acyl or sulfonyl

groups in the presence of a mild base. Indeed, the direct preparation of pentaprotected 2,6-*O*-dibenzoyl *myo*-inositol orthoformate is reported in a one-pot proceedure.[79] However, in order to establish a method for oxidation of the 4-*C*, robust protecting groups which would be stable to a wide range of conditions are required. Acyl and sulfonyl groups are not stable to the strongly basic/nucleophilic conditions which may be needed, for example to effect a Wittig reaction. As a result, the base and largely acid stable benzyl ether was chosen as the permanent 2- and 6-*O* protecting group. Since dibenzylation of inositol orthoformate reportedly yields a 1:5 mixture of 2,4- and 4,6-*O*-diethers favouring diaxially substituted **30**,[82] a temporary protecting group on the 4-*O* would provide the most reliable method for selectively generating the desired 2,6-*O*-dibenzyl isomer. Gigg *et al.*[83] reported the preparation of a range of allyl and benzyl ethers of *myo*-inositol, using the allyl group as a temporary regiodirecting protecting group, which can be removed once the benzyl ethers have been introduced, by isomerisation using ^{t}BuOK in DMSO, followed by mild acidic hydrolysis.[84] Billington *et al.*[76] demonstrated that this difference in stability can be exploited to prepare 2,6-*O*-dibenzyl tetrol from *myo*-inositol orthoformate, Figure 2.8

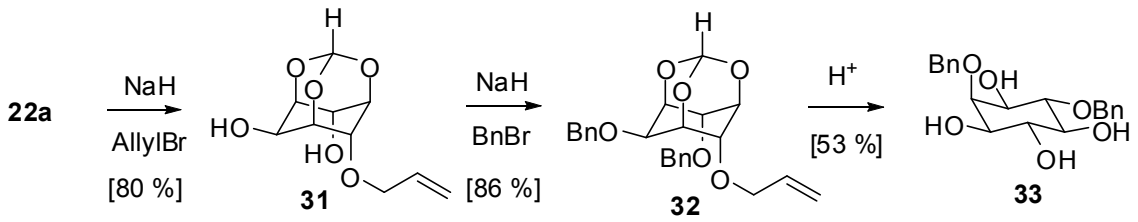


Figure 2.8: Billington's use of 4-*O*-allyl orthoformate to prepare 2,6-*O*-dibenzyl tetrol[76]

It was proposed that this protecting group strategy be used to isolate the 4-*O*H. However, both Billington and Gigg use acidic conditions to remove the isomerised allyl group which effects simultaneous removal of the orthoformate.[85] It will therefore be necessary to employ alternative conditions in order to selectively cleave the temporary 4-*O*-allyl ether in the presence of the 2- and 6-*O*-benzyl ethers and the 1,3,5-*O*-orthoester. For example, the basic removal of a prop-2-enyl ether from an acid sensitive carbohydrate derivative is reported using 4 % aq. $KMnO_4$ in methanolic NaOH.[86]

2.3 Oxidation of the 4-OH and introduction of an alkyl group

Once the 4-*O*H has been isolated it must be oxidised to permit introduction of the *C*-alkyl group. It has been reported that a free inositol orthoester hydroxyl may be oxidised to the inosose, using Swern's method.[87,88] Oxidation of either the 2-*O*H[70,82,88,89] or the 4-*O*H[90] are reported. In some cases, upon oxidation of the 2-*O*H, the observed product is a mixture of the ketone and the *gem*-diol.[5,90] However, the ketone can be isolated by azeotropic removal of water in toluene. This ease of hydration may be attributed to the rigid adamantane structure straining the 120 º sp^2 carbonyl carbon bond angle.[91] Upon oxidation to the inosose one stereocentre and the *myo*-

geometry are lost. Therefore introduction of the alkyl group must be accompanied by regeneration of the *myo*-geometry at the 4-*C*. These two steps can be carried out either simultaneously or stepwise, Figure 2.9.

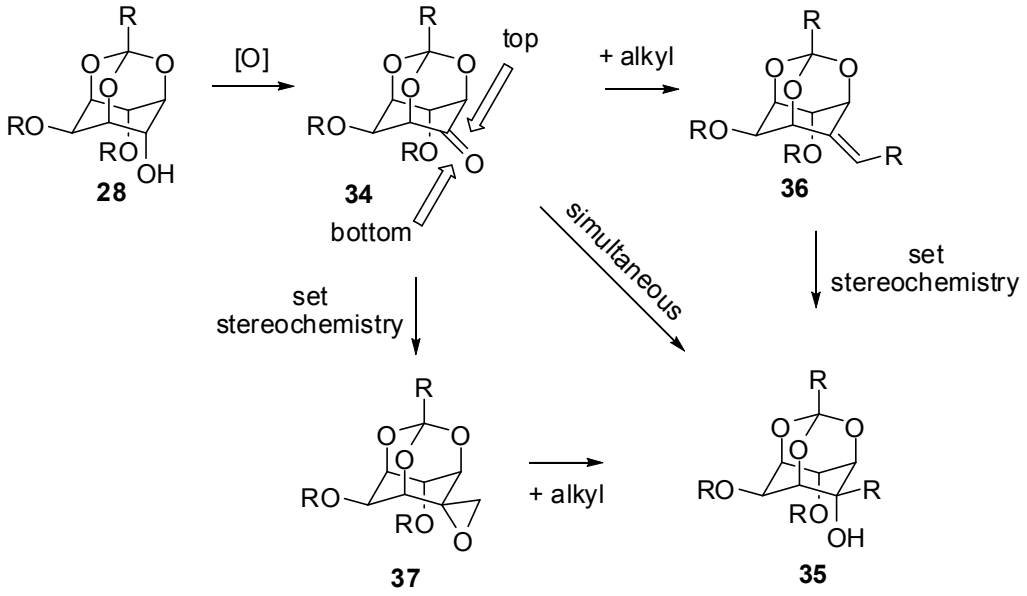


Figure 2.9: The simultaneous or stepwise introduction of 4-*C*-alkyl group

For 4,6-*O*-dibenzyl inos-2-ose and 4,6-*O*-ditosyl inos-2-ose, **39**, reduction with $NaBH_4$ is reported to proceed entirely stereoselectively from the top face[‡] of the inos-2-ose, generating a *scyllo*-geometry product,[70,89] **40**, Figure 2.10a.

[‡] Throughout this thesis the inositol 1,3,5-*O*-orthoesters are consistently drawn in a single orientation, with the orthoester situated above the inositol ring, as illustrated in Figure 2.9, **34**. Therefore references to reagents which approach from or deliver to the top-face describe those which approach from the same face of the inositol ring as the orthoester. Those described as approaching from or delivering to the bottom face approach from the opposite face of the inositol ring to the orthoester protecting group.

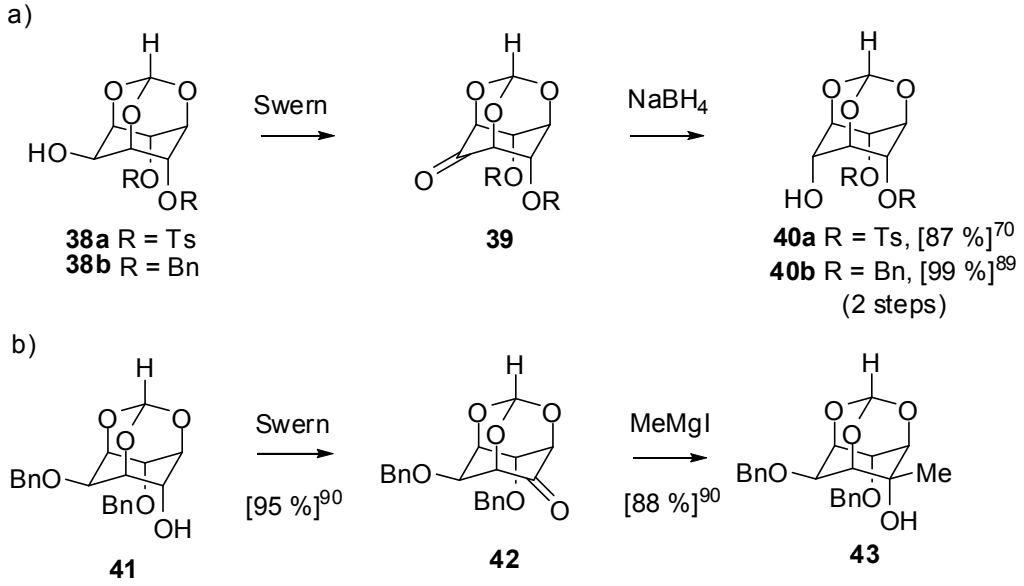
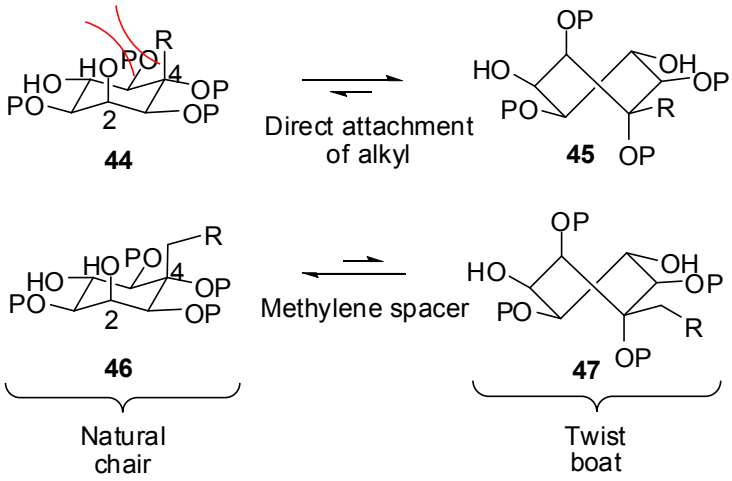


Figure 2.10: a) 2-*O*H oxidation to the inos-2-ose and reduction to the *scyllo*-2-*O*H[70,89]; b) 4-*O*H oxidation to the inos-4-ose and methyl delivery to regenerate the *myo*-4-*O*H and introduce a 4-*C*-methyl substituent[90]

Both 4,6-*O*-dibenzyl inos-2-ose and 2,6-*O*-dibenzyl inos-4-ose have been treated with methylmagnesium iodide to prepare the corresponding 2- and 4-*C*-methyl derivatives.[90] In both cases methyl addition occurred from the top face of the inososе. Therefore for inos-4-ose **42** the original *myo*-geometry was regenerated, **43**, Figure 2.10b. The addition of larger alkyl groups to inososes has not been reported.

While these methods simultaneously reintroduce the *myo*-stereochemistry upon alkyl addition, thereby minimising the number of synthetic steps, the stereochemical outcome depends on the incoming alkyl group, and must be verified in each case. Furthermore, introducing alkyl groups in this manner attaches them directly to the cyclohexane backbone. It was postulated that this would cause conformational strain in the final products as larger *C*-alkyl groups were introduced. Both Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 are known to occupy chair conformations in which a 4-*C*-alkyl substituent, replacing the 4-*H*, would occupy an axial position. It was thought that 1,3-diaxial interactions, more so with bulkier alkyl groups, might cause ring flipping or skewing to a twist boat to alleviate trans-annular strain (**44** → **45**). The natural conformation is essential for binding to the protein as it presents the constellation of charged phosphate esters to the corresponding positively charged residues in the binding pocket in a precise relative geometry. Therefore it was proposed that a methylene spacer be incorporated between the alkyl group and inositol ring to place larger secondary, tertiary and aryl groups further away from the cyclohexane backbone, allowing them to point out radially **46**, Figure 2.11.



P = phosphate, R = bulky

Figure 2.11: Addition of a methylene spacer preserves the natural configuration in the 4-*C*-alkyl IP_n analogue.

The alternative to the reported[90] direct addition is a stepwise approach, in which the stereochemistry can be set either prior to, or after alkyl group insertion, Figure 2.9. In the latter case the stereochemistry is again dependent on the incoming alkyl group, and may change for each new derivative. In the former, the stereochemistry is defined prior to alkyl addition and the stereochemistry of alkylation therefore does not require verification for each new substituent. Epoxides are reactive intermediates with defined stereochemistry, which should undergo nucleophilic ring-opening to introduce the alkyl group, simultaneously including a methylene spacer. Insertion of a methylene group to inos-4-ose **48** to generate an epoxide can result in either the desired *myo*-inositol *exo*-methylene oxide, **49**, by methylene insertion to the top face of the inosose, or the unwanted *epi*-inositol *exo*-methylene oxide, **50**, by insertion to the bottom face, Figure 2.12.

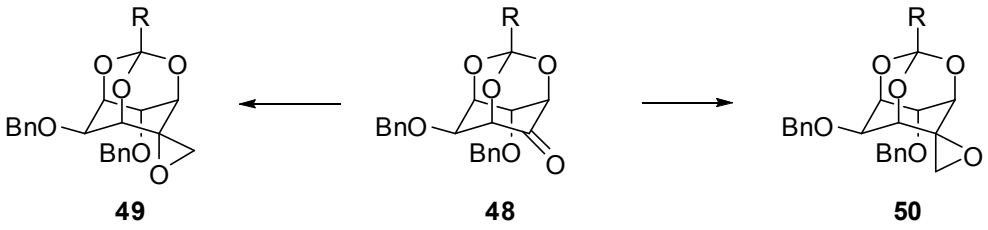


Figure 2.12: Pre-defining the 4-*C*-stereochemistry by conversion to the *exo*-methylene oxide can generate two geometries, *myo* or *epi*.

The preparation of epoxides is a well developed and highly useful tool for organic synthesis.[92] Reported methods of epoxidation include direct insertion of methylene into the inosose using sulfur ylides,[93] chloromethyl lithium[92e] or diazomethane[92c-d,94]. Alternatively conversion of the ketone to an olefin using Wittig conditions, followed by oxidation with *m*CPBA[92a] or H_2O_2[92b] are also reported to generate the corresponding epoxide. In the latter case, although conversion of

the inososе to the olefin is reported in good yield,[88] in this laboratory subsequent oxidations proceeded without the required stereoselectivity (see section 2.6). It was therefore hoped to generate the epoxide directly from the inos-4-ose, using the readily available sulfur ylides.[93]

Corey and Chaykovski first reported dimethyloxosulfonium methylide and dimethylsulfonium methylide as methylene transfer reagents, forming epoxides by insertion of methylene into a ketone double bond.[93] Dimethyloxosulfonium methylide, prepared by the reaction of trimethyloxosulfonium chloride or iodide with sodium hydride, was observed to form only new equatorial carbon-carbon bonds **52** with cyclohexanone derivatives such as **51**, Figure 2.13.

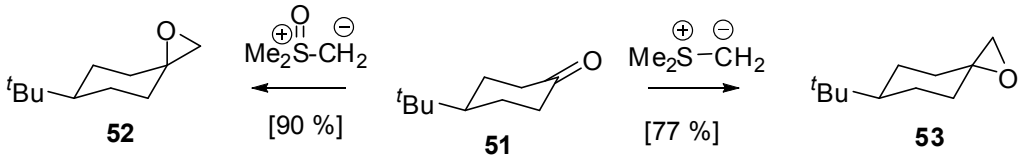


Figure 2.13: Methylene insertion with dimethylsulfoxonium and dimethylsulfonium ylides[93]

In contrast, the corresponding ylide prepared by addition of sodium hydride to trimethylsulfonium iodide was reported to preferentially generate axial carbon-carbon bonds **53**, when added to ketones such as **51**, (observed ratio **53**-**52**, 83:17)[93] Figure 2.13. The reaction of 5-substituted adamantan-2-ones, which are structurally similar to the inososе orthoesters, with both methylides has also been reported to occur in good yield, generating a mixture of *exo*-methylene oxides with limited stereoselectivity.[95] Furthermore, sulfur ylide chemistry has been successfully applied to inososе orthoformates by Paquette *et al.*[96] to generate *exo*-methylene oxides selectively at either one, two or all of the 2-, 4- and 6-*O* for use as lithium ion chelaters. Using dimethylsulfoxonium methylide, they report exclusive delivery to the top (equatorial) face of the inososе to form **55**. They also demonstrated, that upon addition of further ylide, the initially formed *exo*-methylene oxide could be expanded to the oxetane **56** by a second methylene insertion, Figure 2.14.

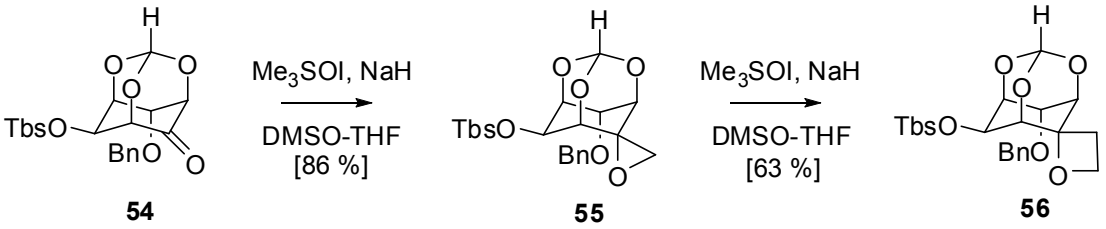


Figure 2.14: Addition of dimethylsulfoxonium methylide to the inos-4-ose generates the *exo*-methylene oxide.[96]

It was hoped that the same face selectivity would be observed for the proposed 2,6-*O*-dibenzyl inos-4-ose, to generate the *myo*-geometry *exo*-methylene oxide. Once an *exo*-methylene

oxide has been introduced, this intermediate can be opened with a nucleophile. There is no literature precedent for such reactions on the inositol framework, although this transformation is extensively covered elsewhere due to its utility in the synthesis of various natural products. For example, the *exo*-methylene oxide **57** prepared from adamantanone was quantitatively opened at the less hindered carbon using *p*-methoxyphenylmagnesium bromide to generate **58**,[97] Figure 2.15.

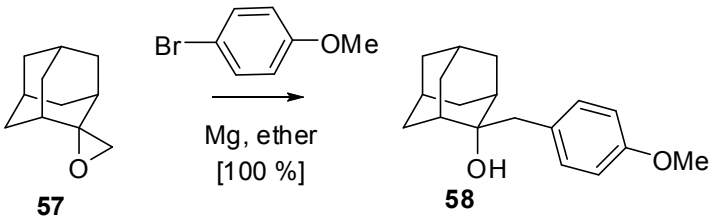


Figure 2.15: Nucleophilic opening of an *exo*-methylene oxide by *p*-methoxyphenylmagnesium bromide.[97]

Further study of the reaction of adamantyleneoxirane **57** with organometallic reagents produced varying results.[98] While addition of phenyl lithium generated the *C*-benzyl derivative **59** in good yield, other reactions were subject to rearrangement or side product formation. For instance the reaction of epoxide **57** with MeMgI and phenylmagnesium bromide generated predominantly iodomethyl **60** and carbinol **61**, Figure 2.16.

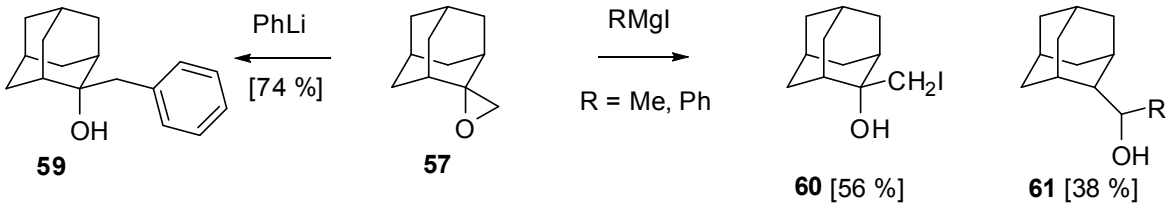
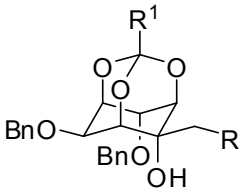


Figure 2.16: The reaction of adamantyleneoxirane with alkylating reagents[98]

Commercial solutions of both Grignard reagents and alkyl lithiums are readily available, and include primary, secondary, tertiary and aryl groups which should allow the introduction of a range of substituents. However, the generation of unwanted side products increases with bulkier nucleophiles and more sterically constrained ketones and epoxides. This has led to modifications of these reactions including catalysis by addition of copper iodide.[99] The addition of copper(I) to phenylmagnesium chloride improved the yield of *trans*-2-phenyl-cyclohexanol from cyclohexene oxide from 3 to 82 %. However, this improvement is not universal as the same conversion with benzylmagnesium chloride did not benefit at all from copper(I) catalysis.

An alkyl group larger than the methyl reported thus far[90] may be needed in order to generate a steric clash which prevents binding of a *C*-alkyl IP_n headgroup to the wild-type lipid binding proteins. The development of a reliable route for the introduction of an alkyl or aryl group of varying size by nucleophilic substitution of the *exo*-methylene oxide is therefore a major goal of the synthesis. Therefore it is important to explore the chemistry of appending varying shapes and sizes of bump onto the ring, Figure 2.17.



62

R = primary alkyl: H (known), methyl, *n*-butyl
= secondary alkyl: *iso*-propyl
= tertiary alkyl: *tert*-butyl
= aryl: phenyl, benzyl, napthyl

R^1 = orthoester head group

Figure 2.17: The proposed target library of 4-*C*-alkyl compounds

Since the bumpy lipid head groups will ultimately need to dock with point mutants of lipid binding proteins, this implies that the bump should be of comparable size and shape to the lost amino acid side chain to produce a specific bump-hole pair, Figure 2.18.

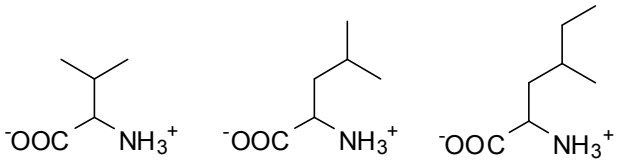
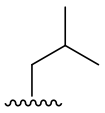
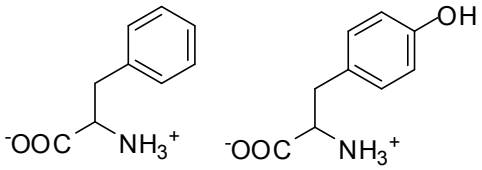
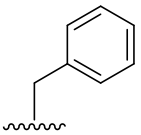
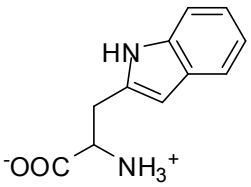
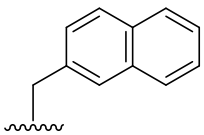
Amino acid	*C*-alkyl equivalent
 Valine Leucine Isoleucine	
 Phenylalanine Tyrosine	
 Tryptophan	

Figure 2.18: Amino acids and their comparable *C*-alkyl substituents

Therefore in addition to alkyl groups, aryl groups such as phenyl, benzyl and 2-naphthyl are good target 4-*C*-alkyl substituents, Figure 2.17. The limitations for manipulation of the 4-*C*-alkyl groups will be explored during the subsequent protecting group manipulations, phosphorylation and deprotection steps. For example, larger alkyl and aryl groups may be prone to elimination either during orthoester unblocking or after phosphorylation because unconstrained

flexing of the ring may allow anti-periplanar relationships of suitable protons and the 4-*O*-phosphotriester to arise.

2.4 Removal of temporary protecting groups to generate both IP_3 and IP_4 derivatives.

A major advantage of the orthoester protecting group is the number of different ways in which it can be selectively cleaved to expose one, two or all of the underlying hydroxyls, accessing a range of products from a single precursor. Three distinct methods of manipulating the orthoester have been explored in the literature: Reduction with diisobutylaluminium hydride (DIBAL-H), methyl insertion into the C-O bond using trimethylaluminium or Grignard reagents, and acidic hydrolysis.

Acidic hydrolysis, followed by removal of the intermediate acyl ester, liberates the 1-, 3- and 5-*O*H. In the case of the 4-*C*-alkyl derivatives, **62**, this should generate the 1,3,4,5-tetrol required for the preparation of 4-*C*-alkyl IP_4. Acidic hydrolysis of orthoformates has been reported using either TFA-H_2O (4:1)[78,100] or HCl in MeOH at reflux.[56,101] During acidic hydrolysis of the orthoformate the presumed intermediate formate ester is spontaneously hydrolysed under the reaction conditions to generate the free hydroxyl of triol **64**. In contrast, larger alkyl groups at the orthoester bridgehead generate relatively stable acyl esters upon acidic hydrolysis (**65**), which can be removed by nucleophilic attack to liberate the final triol **64**, Figure 2.19.

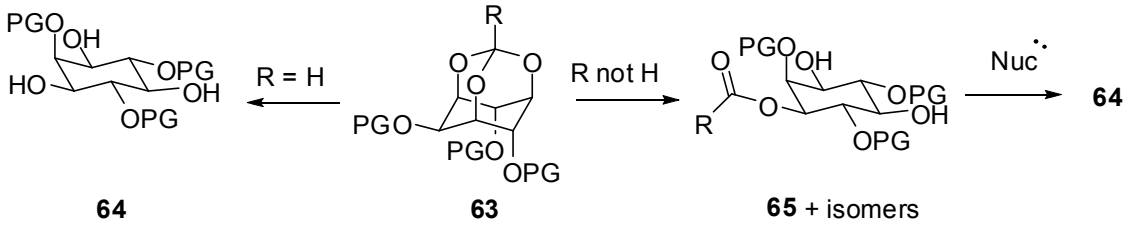


Figure 2.19: Acidic hydrolysis of the orthoester

Potter *at al.*[71] used acidic hydrolysis of the orthoacetate to generate a separable mixture of the 1- and 3-*O*-acetate esters in their preparation of natural Ins(1,4,5)P_3. The same group prepared the 1 and 3-*O*-benzoate esters by acidic hydrolysis of 2,6-*O*-dibenzyl orthobenzoate with 1.0 M HCl in reluxing ethanol (1:2).[73] No 5-*O*-benzoate ester was isolated under these conditions and the mixed benzoate esters could be removed to generate the 1,3,4,5-tetrol. Alternatively, the 1-*O*-ester was used to prepare PtdIns(3,4,5)P_3, since it differentiated the 1-*O* for coupling to phosphatidate from the three phospho mono-esters.[73] These results suggest that the 2- and 6-*O* benzyl ether permanent protecting groups will be unaffected by complete removal of the orthoester.

The orthoester employed for the preparation of 4-*C*-alkyl derivatives is required to withstand the conditions needed to introduce the 4-*C*-alkyl group, but then be selectively removed

without destroying this new tertiary centre, in order to permit phosphorylation. The choice of substitution at the orthoester bridgehead can be used to influence the stability of the orthoester.[102] Bouab *et al.*[103] studied rates of hydrolysis of 2,4,10-trioxaadamantanes, **66**. For these compounds, very reminiscent of the inositol orthoesters, they report that substitution at the orthoester bridgehead reduces the rate of hydrolysis. This is because the rate determining step requires a ring-flip of the cyclohexane ring to the boat conformation **70** to allow water to bind to the carboxonium ion. As the carboxonium ion is increasingly stabilised by larger groups the rate of addition of water to **70** is reduced as the size of R increases,[103] Figure 2.20.

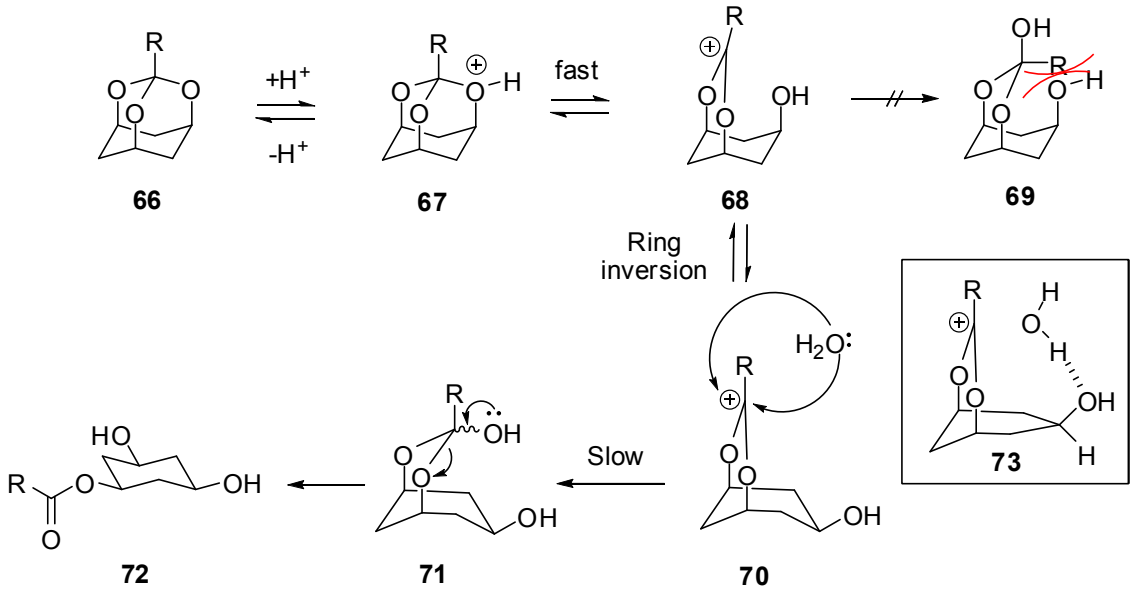


Figure 2.20: The rate determining step of orthoester hydrolysis requires a ring-flip to permit the addition of water to the carboxonium ion.[103]

Bouab *et al.*[103] suggested that the key intermediate in this transformation was **73**, in which addition of water to the carboxonium ion is simultaneous with ring-flip from the chair to the boat conformation. However, it seems unlikely that such a high energy transient species could undergo coordination of water before ring-flip to a more stable intermediate.

An additional protecting group on the 3-*O* is required to prepare the desired 2,3,6-*O*-protected triols for 4-*C*-alkyl IP_3 analogues. In contrast to acidic hydrolysis, reduction of the orthoester with limited DIBAL-H selectively generates a bridging acetal, **75**. First reported by Gilbert and Holmes,[104] two equivalents of DIBAL-H are required to effect reduction of the orthoformate to the formylidene acetal. Correspondingly, reduction of the orthobenzoate with limited DIBAL-H generates the bridging 1,3-*O*-benzylidene acetal in good yield.[105] Delivery of hydride to *myo*-inositol orthoesters occurs selectively by initial Al coordination to the relatively unhindered 5-*O*.[106] Access to the 1 and 3-*O* is more obstructed, presumably due to the equatorial 2-*O*Bn. Upon second hydride delivery the 1,3-*O*-acetal would presumably be further reduced to a 1- or 3-*O*-benzyl ether **76a** and **b**, Figure 2.21.[107]

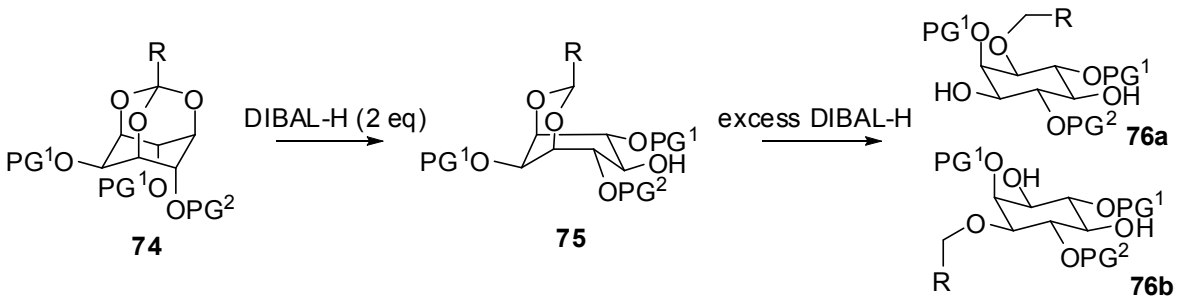


Figure 2.21: DIBAL-H insertion into the 5-*O*-C bond, followed by a second insertion to generate a benzyl ether

Trimethyl aluminium and Grignard reagents are reported to cleave orthoesters preferentially at the 1(3)-*O*-C bond to generate an acetal such as **77**, due to chelation of the metal in the *myo*-1,2,3-*O*-trioxa pocket.[104] This regioselectivity complements that of DIBAL-H reduction at the 5-*O*, Figure 2.22.

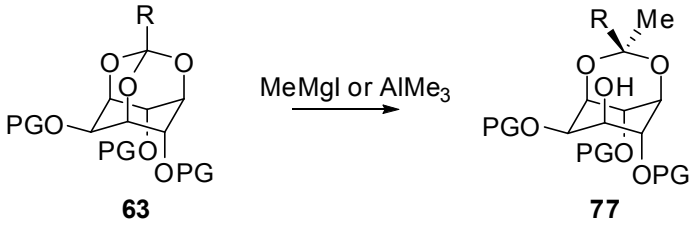


Figure 2.22: Regioselective opening of orthoesters by trimethyl aluminium or Grignard reagents

This interpretation of the regioselectivity was corroborated by the observation that in the absence of an equatorial 2-*O*, such as for a *scyllo*-derivative, the orthoester is not cleaved by Grignard reagents.[108] The proposed mechanisms for the differing sites of cleavage with DIBAL-H or MeMgBr and subsequent addition to the oxonium ion are shown in Figure 2.23.[106]

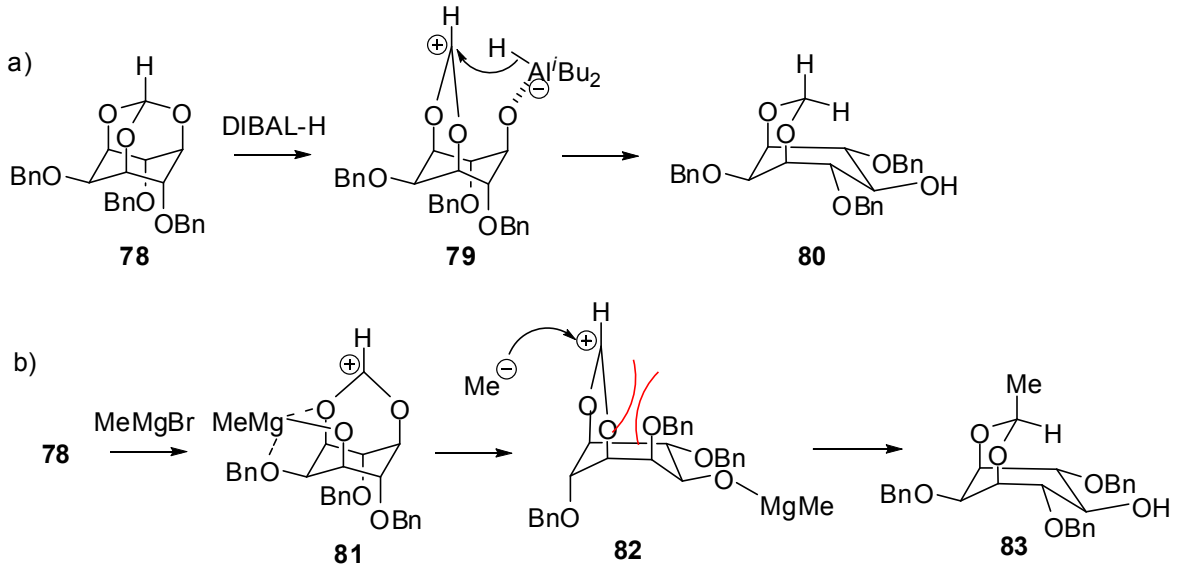


Figure 2.23: Proposed mechanisms of orthoester cleavage with DIBAL-H or MeMgBr.[106]

Upon treatment of an orthoester with methyl Grignard, the methyl group was delivered to the opposite face of the bridgehead carbon, relative to the site of cleavage. This face selectivity is reported to result from steric hindrance by the pseudo-axial 2-*O*Bn which results from a conformational change of intermediate **81** to **82**, Figure 2.23b.[106] Treatment of the orthoformate with larger Grignard reagents, such as phenylmagnesium bromide is also reported, generating a 1,3-*O*-benzylidene acetal from an orthoformate.[108] The addition of excess Grignard reagent to the orthoformate effects a second alkyl delivery, cleaving the remaining 1(3)-*O*-C bond to generate the 5-*O*-protected diol. The preference for generating 5-*O*-alkyl derivatives upon attack with Grignard reagents make this route less attractive than the alternative DIBAL-H reduction for the direct preparation of the 4-*C*-alkyl IP_3 triols.

As a result of the selective methods reported for deprotection of orthoesters, it was postulated that manipulation of one orthoester would allow access to both 4-*C*-alkyl-IP_3 and -IP_4 derivatives. The orthobenzoate, although less widely used in the literature to date, appears to have the potential to generate both groups of compounds. 4-*C*-Alkyl IP_4 derivatives could be accessed by acidic hydrolysis of the orthobenzoate and subsequent removal of the benzoate ester. It was hoped that the orthobenzoate would be less labile than the orthoformate during alkyl introduction, but that it would still be selectively removed in the presence of the permanent benzyl protecting groups, under acidic hydrolysis. The orthobenzoate also has the advantage that 1-*O*-benzoate ester may be isolated for PIP_3 synthesis, allowing this route to be used in the later generation of the corresponding 4-*C*-alkyl phosphatidyl lipids. 4-*C*-Alkyl IP_3 derivatives may be accessed by reduction of the orthobenzoate to a benzyl ether using DIBAL-H. Regioselective total reduction of 2,6-*O*-dibenzyl orthobenzoate to generate the 3-*O*-benzyl ether would generate the necessary triol for IP_3 analogues. To favour this isomer over the unwanted 1,2,6-*O*-tribenzyl ether, it is necessary

to influence the regioselectivity of second hydride delivery to the 1,3-*O*-benzylidene acetal. This would be beneficial both in the synthesis of natural IP_3 and the preparation of 4-*C*-alkyl IP_3 analogues.[107] If the respective 4-*C*-alkyl triols and tetrols can be prepared, these will then be ready for phosphorylation and subsequent deprotection.

2.5 Phosphorylation of polyols and deprotection of the phosphate esters and hydroxyls

Phosphorylation of polyols is extensively reported in relation to IP_n and PIP_n synthesis.[109-113] In addition to the usual difficulties of effecting multiple phosphorylations while avoiding contamination with cyclic phosphotriester by-products,[114] the 4-*C*-alkyl tertiary hydroxyl must be phosphorylated to generate the desired analogues. Polyphosphorylation during preparation of inositol phosphates has been reported either a) directly with P(V) reagents (in which the phosphorous atom is already in the correct oxidation state), or b) indirectly using P(III) reagents which generate an intermediate phosphite triester that must be subsequently oxidised to the required phosphate triester.

The P(V) reagents are more stable (as are the resultant phosphate triesters), than their P(III) counterparts which are more sensitive to attack by nucleophiles such as water. However, the preparation of inositol phosphates usually requires phosphorylation of multiple secondary centres, often adjacent to one another, and phosphorylation with the less reactive P(V) reagents has sometimes proved difficult.[54,115] There are reports of P(III) reagents succeeding in phosphorylating vicinal hydroxyls where P(V) reagents have failed.[114]

Phosphorylation of vicinal hydroxyls is problematic because general intermediate **84** must form transiently with a phosphate or phosphite triester adjacent to a free hydroxyl, Figure 2.24.

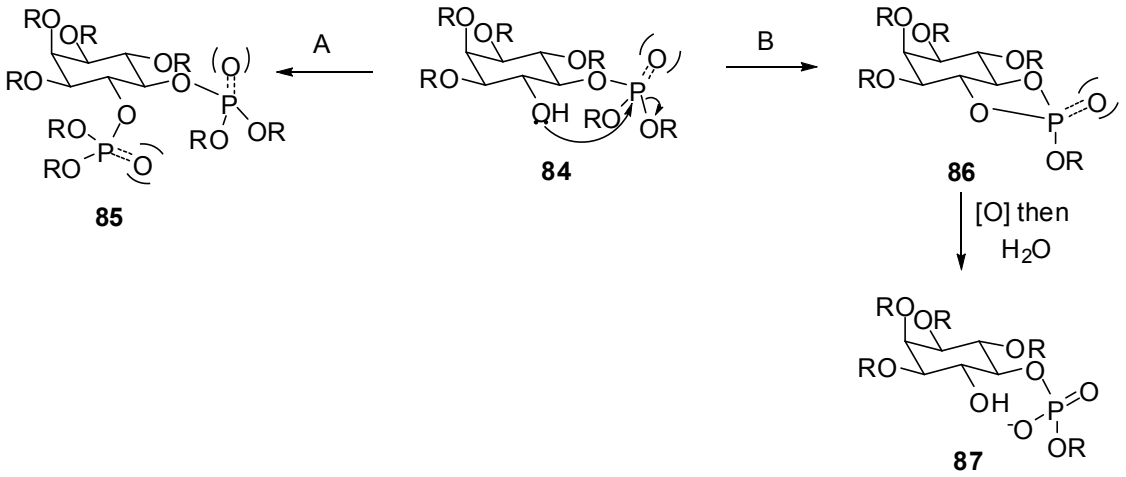
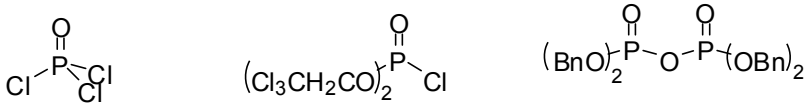


Figure 2.24: Phosphorylation of vicinal diols can suffer from competing cyclisation reactions

From this intermediate there are two possibilities: Either second phosphorylation or phosphitylation of the free hydroxyl, to generate **85**; or attack of the free hydroxyl on the adjacent phosphate/phosphite triester to form cyclic **86**. These cyclic species are hydrolysed during work up to generate **87**, reducing the yields of phosphorylation reactions. Therefore the key to polyphosphorylation is to ensure that reaction pathway A happens faster than B. The P(V) phosphates are considerably more stable than the corresponding P(III) phosphites, so the rate of transesterification of these intermediates is perhaps 10 times slower. This means that they are slower to form cyclic species. However the steric bulk means that the rate of phosphorylation of the adjacent hydroxyl is also slow. For the more reactive P(III) phosphites, although more easily attacked by the adjacent hydroxyl to generate cyclic products, the corresponding rate of phosphitylation of the second hydroxyl is much faster than the P(V) reagents.

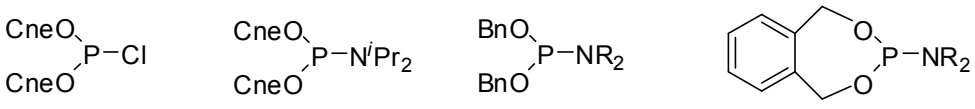


88 **89** **90**, TBPP

Figure 2.25: P(V) reagents

Phosphorous(V)oxychloride **88**, Figure 2.25, was first used in 1857[54] and more recently[115] to effect step-wise phosphorylation of a vicinal 4,5-diol. Reagent **89**, could only mono-phosphorylate the diol, generating a mixture of products, but this was successfully phosphorylated with $POCl_3$. The most commonly reported P(V) reagent is tetrabenzyl pyrophosphate (TBPP, **90**) which is commercially available.[112,116] In the first reported synthesis of Ins(1,3,4,5)P_4, Billington and Baker[85] use TBPP with sodium hydride in THF to effect tetraphosphorylation of the 1,3,4,5-tetrol, in 66-70 % yield in the presence of a catalytic quantity of imidazole or 18-crown-6. Efficient phosphorylation with TBPP requires conversion of the hydroxyls into alkoxides.[117] These strongly basic conditions could be problematic with compounds containing sensitive functionalities, leading to decomposition.

Despite their instability towards moisture and oxidation, P(III) reagents are now widely used in inositol polyphosphate synthesis, Figure 2.26.

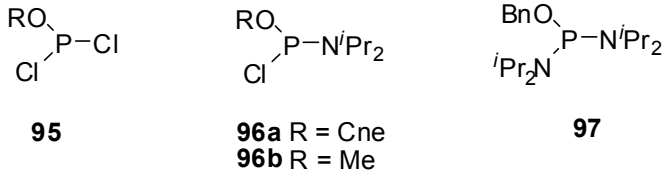


91, Cne = CH_2CH_2CN **92** **93a** R = Et **93b** R = ^{i}Pr **94a** R = Me **94b** R = Et

Figure 2.26: P(III) reagents

For example, di(2-cyanoethyl)phosphorochloridite **91** was used to construct all the phosphoryl centres in the Gaffney and Reese synthesis of PIP_3.[113] The P(III) phosphoramidites have also been used extensively as they are more stable to hydrolysis than the corresponding chloridites,[109] but are activated by weakly acidic 1-*H*-tetrazole. Di(2-cyanoethyl) phosphoramidite **92** has been frequently employed, including Reese and Ward's first synthesis of IP_3.[118,119] Alternative phosphoramidites include those protected with benzyl esters; *N,N*-diethyl- and *N,N*-diisopropyldibenzyl phosphoramidite, **93a** and **b**. The latter was preferred in the preparation of both enantiomers of Ins(1,3,4,5)P_4[120] due to its easier purification by column chromatography[110] and is now commercially available from *Aldrich*. More recently, *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane **94b** has been used in the phosphitylation of multiple hydroxyls.[111] This less sterically demanding reagent is easily prepared by the reaction of hexaethylphosphorous triamide with 1,2-di(hydroxymethyl)benzene.[121]

The requirement for the formation of a 1-phosphodiester during phospholipid synthesis, and the desire to prepare thio-analogues of phosphate esters for biological studies, led to the development of bifunctional phosphitylating reagents, Figure 2.27.



95 **96a** R = Cne **96b** R = Me **97**

Figure 2.27: Bifunctional phosphorylating reagents

As a result of the differential reactivities of the two chlorines of phosphorodichloridites **95**, they can be reacted with two different partners to form a protected phosphodiester bridge after oxidation, which is useful in both phosphoinositide and oligonucleotide synthesis. 2-Cyanoethoxy(diisopropylamino)chlorophosphine and the corresponding methoxy derivative **96** have been used in the preparation of phosphodiester linkages and also phosphorothioate analogues of the inositol phospholipids.[122,123] Phosphitylation using (benzyloxy)bis(diisopropylamino) phosphine **97** with 1-*H*-tetrazole and subsequent oxidation was used to prepare stable cyclic phosphates as conformationally restricted inositol phosphate analogues.[124]

Following phosphitylation with a P(III) reagent, the resultant phosphite triester must be oxidised to the phosphate triester. The most effective oxidations employ a one pot procedure without isolating the phosphitylated material, reducing the opportunity for hydrolysis. *m*CPBA[110] and ^{t}BuOOH[125] are the most commonly employed oxidising agents. Yu and Reid[110] reported that they observed cleaner oxidations of dibenzylphosphite triesters with *m*CPBA rather than ^{t}BuOOH in CH_2Cl_2. The use of P(III) reagents to prepare initial phosphite triesters has allowed their facile

conversion to thioylated inositol analogues. Cooke *et al.*[126] followed phosphitylation with di(2-cyanoethyl)phosphorochloridite **91** by oxidation to the phosphorothioates using sulfur in pyridine.

To the best of our knowledge, the phosphorylation of a tertiary centre as required by the proposed 4-*C*-alkyl targets, has not been reported in the literature. It would be expected that this centre will undergo phosphorylation more slowly than the adjacent secondary centres due to increased steric hindrance from the additional alkyl substituent. This may have undesirable effects in terms of side product formation; for example cyclic phosphate formation by attack of the tertiary 4-*O*H on the adjacent 3- and 5-phosphites. Therefore it was expected that a highly reactive P(III) phosphorylating reagent would need to be employed, followed by oxidation with *m*CPBA to generate the phosphate triesters.

Once phosphorylated, to prepare the final inositol phosphates, both the phosphates and alcohols need deprotecting. During deprotection it is essential that the phosphate groups are unblocked prior to the alcohols in order to prevent migration of the protected phosphate esters. Once the phosphate esters are deprotected, the negative charge suppresses nucleophilic attack by neighbouring hydroxyls and hence prevents migration from occurring. Therefore, as long as the pH is maintained between 3 and 10, the alcohols may be unblocked without risk of migration. If possible it is convenient to effect a global one step deprotection, by generating a fully protected analogue with only one protecting group.[120] This avoids further manipulations of highly charged, polar species. The benzyl protecting group has frequently been chosen for such a deprotection as it can be removed under neutral conditions using hydrogenolysis.[85] The benzyl phosphate esters are considerably more labile (believed to be >1000 fold) than the benzyl ethers on the 2- and 6-hydroxyls which should ensure that the phosphate esters are deprotected first.[127] Considering that for the 4-*C*-alkyl analogues the combination of an electron withdrawing phosphotriester and 4-*C*-alkyl substituent on a tertiary centre may make these substrates susceptible to β-elimination, the mild conditions of hydrogenation could prove essential to the success of total deprotection.

It was anticipated that phosphorylation of the 4-*C*-alkyl triols and tetrols with *N,N*-diisopropyldibenzyl phosphoramidite, using the method of Yu and Reid,[110] would be sufficient to react with the tertiary hydroxyl. This P(III) species is highly reactive and has performed well in published polyphosphorylations.[110] It also has the advantage that the phosphate protecting groups are benzyl esters, which should permit a one step deprotection to generate the final products.

2.6 Previous work towards the introduction of a 4-*C*-alkyl substituent

During my MChem project, isolation of the 4-*O*H and initial attempts to introduce a 4-*C*-alkyl group were explored.[128] The inos-4-ose was prepared using the following key steps, Figure 2.28.

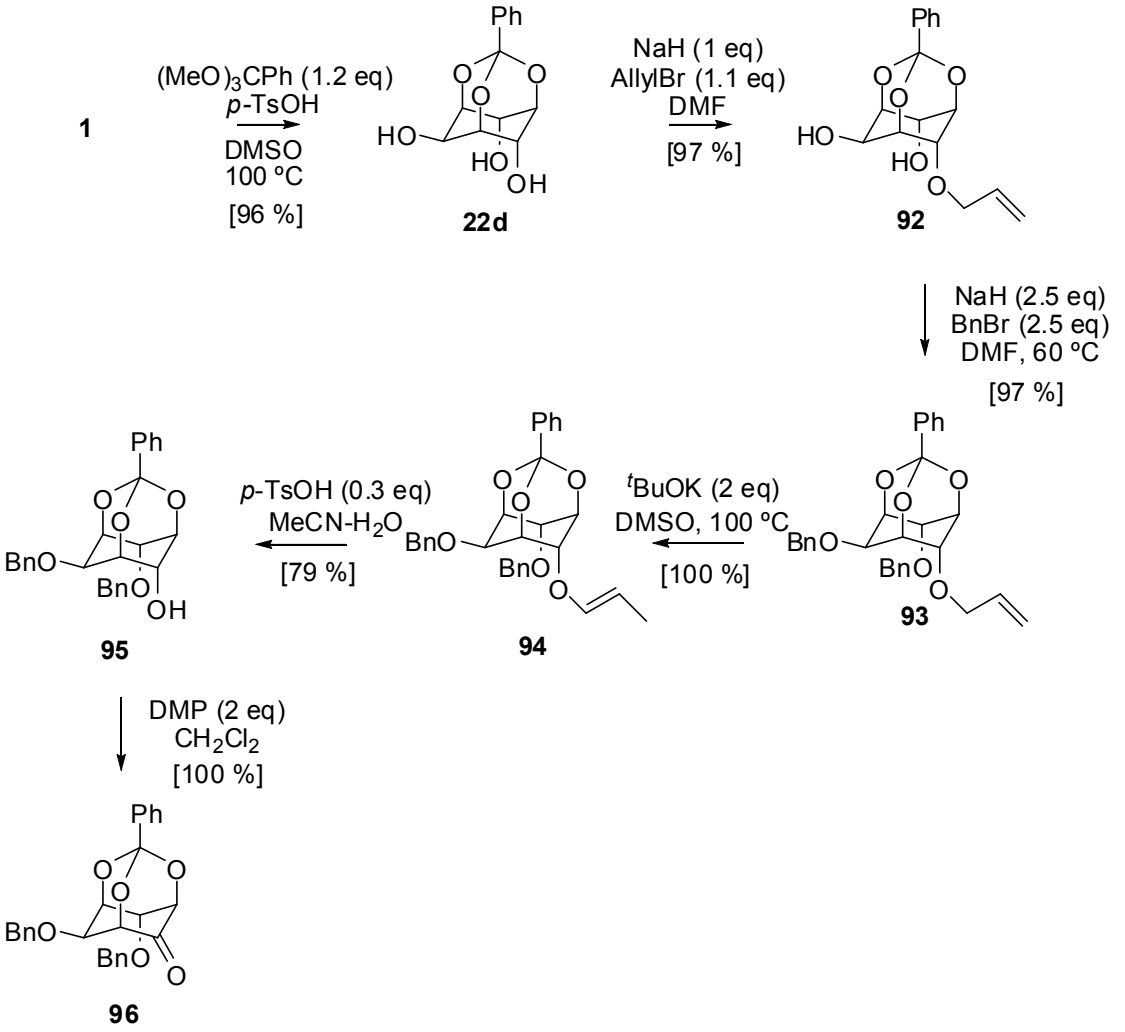


Figure 2.28: Preparation of the inos-4-ose by oxidation of the isolated 4-*O*H

4-*O*-Allyl orthobenzoate **92** was prepared directly from crude orthobenzoate **22d** via deprotonation of the 4-*O*H with sodium hydride, to place a temporary allyl ether on the 4-*O*,[84] followed by flash chromatography on a large (10 g) scale, avoiding the time-consuming reported recrystallisation[73] of orthoester **22d**. Protection of the remaining two hydroxyls as benzyl ethers, then isomerisation of the allyl group with potassium *tert*-butoxide, followed by its removal under acidic conditions, afforded dibenzyl ether **95** with a single free 4-*O*H. This was oxidised to inosose **96** using Dess Martin periodinane (DMP), which gave cleaner results than the corresponding oxidation under Swern conditions.

From the key intermediate **96** conversion to the *exo*-methylene oxide using Wittig conditions, as previously reported for the inos-2-ose,[88] occurred in good yield, Figure 2.29.

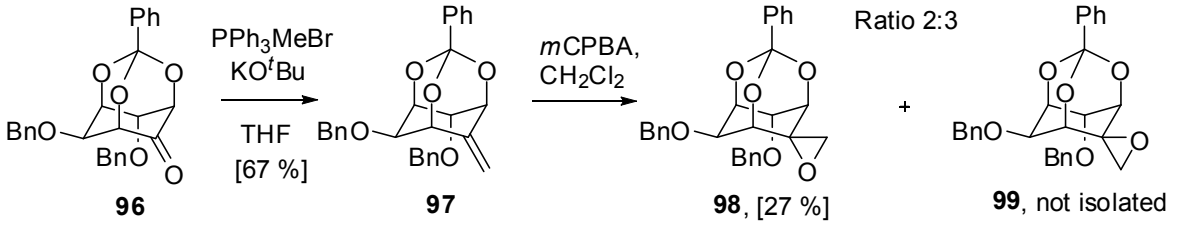


Figure 2.29: Conversion of the inos-4-ose to an *exo*-methyelene and generation of both epoxides upon addition of *m*CPBA

Addition of oxygen to the double bond of *exo*-methylene **97** using *m*CPBA[92a] was not face-selective, despite the 6-*O*-benzyl protecting group which might have been expected to obstruct access to the bottom face of **97**. A separable mixture of the *myo*- and *epi*-inositol *exo*-methylene oxides **98** and **99** were recovered in a ratio of 2:3 *myo*-*epi*, as judged by ^{1}H-NMR of the crude material. It was postulated that the lack of selectivity might derive from a balance of opposing influences: Greater accessibility along a less hindered equatorial approach, but (despite possible obstruction of the axial approach by the 6-*O*-benzyl ether) delivery to the lower face by the 6-*O* hydrogen bonding to the peracid. Reduction of both *exo*-methylene oxides with $LiAlH_4$ to the 4-*C*-methyl derivatives permitted identification of the stereochemistry of methylene addition. The desired *myo*-inositol epoxide was the higher R_f product of the two. These species were easily identified by characteristic double doublets in the ^{1}H-NMR [δ_H 2.95 (1H, d, *J* 4), 2.93 (1H, d, *J* 4) ppm and δ_H 2.89 (1H, d, *J* 4), 2.82 (1H, d, *J* 4) ppm].

It was hoped that a less hindered, free 6-*O*H would direct epoxidation preferentially to the desired bottom face of the *exo*-methylene by H-bonded coordination to *m*CPBA. Therefore **100** was prepared in 5 steps from *myo*-inositol orthobenzoate **22d**, Figure 2.30.

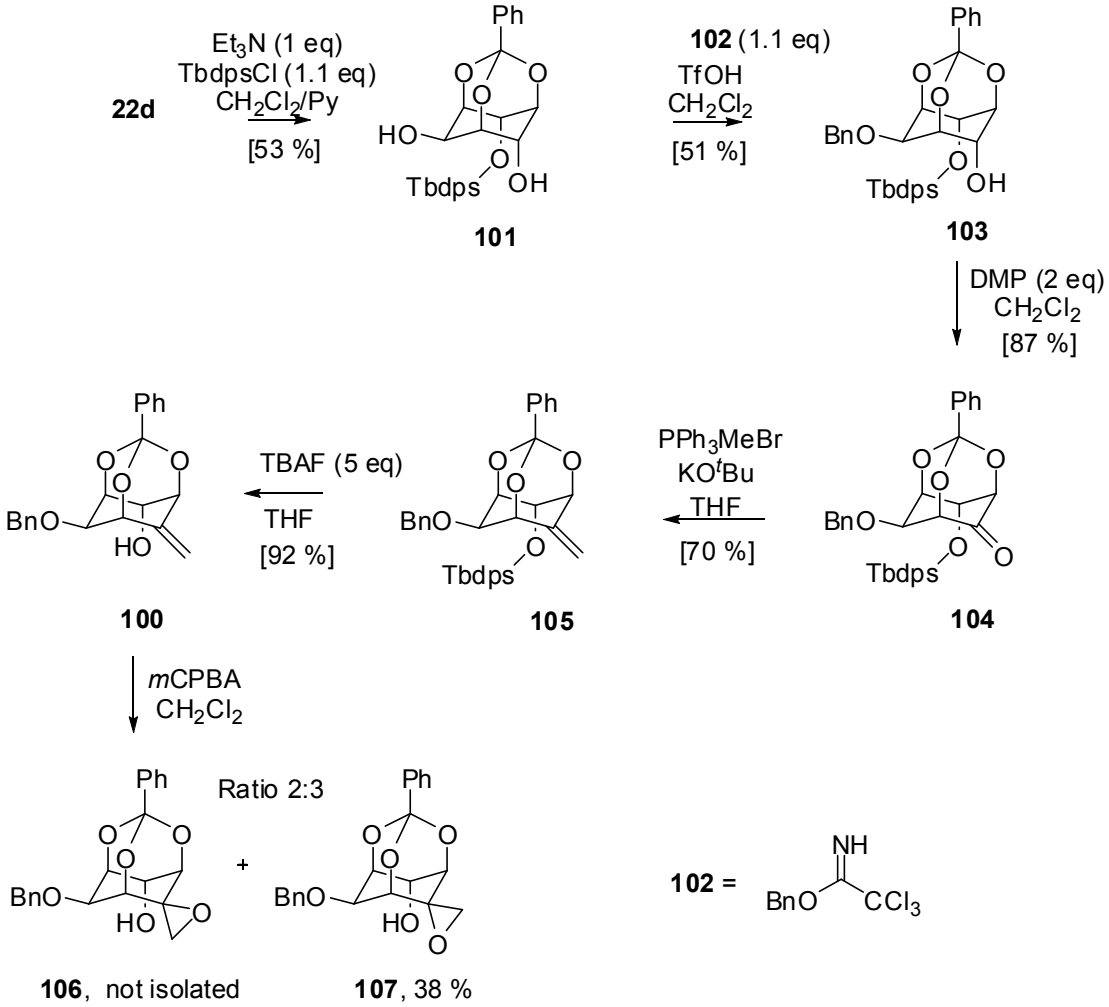


Figure 2.30: Preparation of **100** with free 6-*O*H for coordinated *m*CPBA delivery

The orthobenzoate **22d** was reacted with *tert*-butyldiphenylsilylchloride (Tbdps-Cl) in the presence of triethylamine to selectively afford 6-*O*-Tbdps, **101** (see section 2.2). A benzyl ether was then introduced onto the less hindered 2-*O* using mildly acidic conditions, generating **103** in 51 % yield, with small amounts of the 2,4-*O*-dibenzyl ether also recovered. Oxidation of the free 4-*O*H of **103** to inos-4-ose **104**, followed by conversion to *exo*-methylene oxide **105** was carried out in the same manner as for the 2,6-*O*-dibenzyl ether **96**. The 6-*O*-Tbdps was removed using TBAF to generate **100** with a free 6-*O*H for coordinated delivery of *m*CPBA. Upon addition of *m*CPBA, oxygen delivery to the lower face of the *exo*-methyene was favoured, generating the *myo*-epoxide **107** preferentially, although the ratio of products was only 3:2 as judged by ^{1}H-NMR of the crude mixture. However the reaction was slow suggesting that neighbouring group delivery was not occurring. The increase in desired *myo*-epoxide relative to the epoxidation of **97**, may therefore only be related to the reduced steric hindrance of approach to the bottom face of the *exo*-methylene with the free 6-*O*H.

Following the lack of selectivity observed upon oxygen delivery to the *exo*-methylenes, the use of sulfur ylides to insert a methylene group directly into inososse **96** was investigated. Treatment of **96** with dimethylsulfonium methylide using the methodology of Corey and

Chaykovsky,[93] effected only 20 % reaction, with the remainder recovered as unreacted starting material, Figure 2.31.

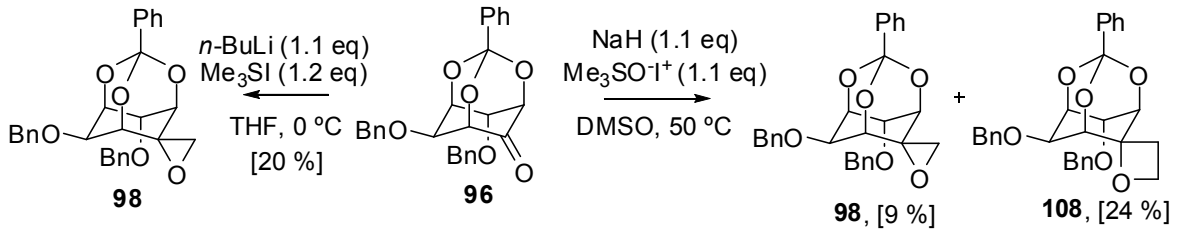


Figure 2.31: Insertion of methylene directly to inos-4-ose using sulfur ylides

In contrast to the observation of Corey, the material recovered from the reaction was the result of equatorial addition to the inosose, generating the *myo*-inositol stereochemistry. Further attempts to generate the *exo*-methylene oxide with dimethylsulfoxonium methylide generated two products, **98** and **108** along with unreacted **96**. *exo*-Methylene oxide **98** and oxetane **108**, resulting from a second insertion of the ylide to epoxide **98**, were recovered in the ratio 1:3 favouring the oxetane, as judged by ^{1}H-NMR of the crude mixture, Figure 2.31. Although both products were the result of the required stereoselective methylene insertion from the top face of the inosose, further investigation was required to control the number of methylene insertions and optimise the reaction yield.

2.7 Summary of synthetic strategy for the preparation of 4-*C*-alkyl IP_3 and IP_4 compounds

The primary objective of the work presented in this thesis was to synthesise *C*-alkyl Ins(1,4,5)P_3 and *C*-alkyl Ins(1,3,4,5)P_4 analogues. The 4-*C* was chosen as an initial target, as it could be easily isolated to permit investigation of alkyl introduction, and the subsequent manipulation, phosphorylation and deprotection steps, within the time available, Figure 2.32.

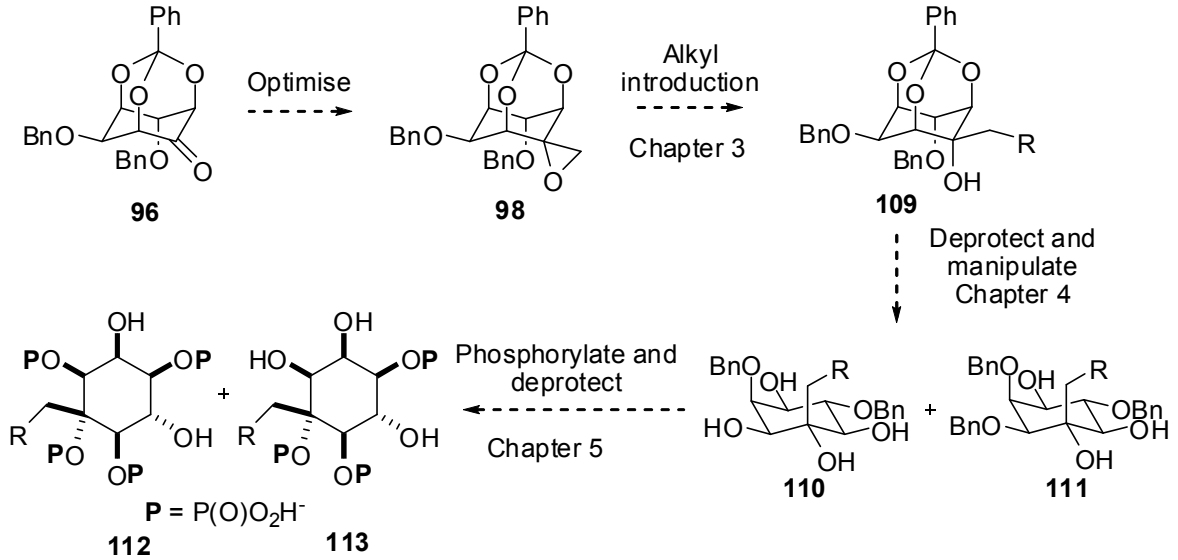


Figure 2.32: Summary of synthetic strategy towards 4-*C*-alkyl IP_3 and IP_4 derivatives

In order to achieve the end targets, four intermediate goals were identified and the latter three form the main body of the experimental work described in this thesis.

a) Protection of *myo*-inositol and isolation of the 4-*O*H had been carried out in the MChem project[128] (see section 2.5). The inos-4-ose was successfully prepared in 6 steps with an overall yield of 71 %. This provided an ideal starting point for further investigations toward the introduction of a *C*-alkyl group.

b) Initial attempts to convert the inos-4-ose to an *exo*-methylene oxide using sulfur ylide chemistry were stereoselective, although optimisation of this transformation was still required. Once prepared, a *myo*-geometry *exo*-methylene oxide could be opened with a nucleophilic carbanion equivalent. Introduction of an alkyl group directly to the inosose has been considered, and although possible, is not the preferred route to the 4-*C*-alkyl compounds due to the likelihood of 1,3-diaxial interactions resulting in distortion of the final products with secondary and tertiary alkyl or aryl groups. These results are discussed in chapter three.

c) Following the introduction of a 4-*C*-alkyl substituent, the temporary protecting groups need to be removed to selectively generate free hydroxyls for phosphorylation. For IP_4 derivatives, the 1,3,4,5-tetrol requires full removal of the orthobenzoate, by acidic hydrolysis to the benzoate ester, followed by ester exchange to the free hydroxyl. For IP_3 derivatives, the orthoester must be cleaved at the 1 and 5-*O*, to generate the 1,4,5-triol. Manipulation of the orthoester is proposed using DIBAL-H. These results are discussed in chapter four.

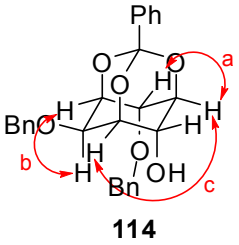
d) The 4-*C*-alkyl triols and tetrols must be phosphitylated and oxidised to generate the fully protected phosphate esters. It was hoped that phosphitylation with *N,N*-diisopropyldibenzyl phosphoramidite, followed by oxidation with *m*CPBA would provide reactive enough phosphitylating conditions, followed by a clean oxidation to the phosphate esters. Subsequent global deprotection of the benzyl phosphate esters and benzyl ethers under H_2 with Pd catalysis should generate the desired 4-*C*-alkyl IP_3 and IP_4 products. These results are discussed in chapter five.

2.8 NMR of the target compounds

A key feature of this thesis is the assignment of regio- and stereochemistry using ^{1}H-, ^{13}C- and ^{31}P-NMR. The six protons around the *myo*-inositol cyclohexane ring provide an invaluable tool for analysis of these compounds by NMR. Each carbon bears a secondary hydroxyl, which deshields these protons and shifts them into the 3-5 ppm region of the ^{1}H-NMR and 60-80 ppm region of the ^{13}C-NMR spectra. In ^{1}H-NMR, the splitting patterns of these protons provide information about the number of neighbouring ring protons and, due to the constrained cyclohexane ring, the spatial relationship of these adjacent protons can be interpreted using the

coupling constants of the multiplets generated by each proton according to the relationship derived by Karplus[129]. Spectra which illustrate the key transformations are included for reference in Appendix D.

The protection of the 1-, 3- and 5-*O*H by an orthoester provides a very rigid, adamantane-like structure and constrains the cyclohexane ring to a single conformation. This was clearly visible in the ^{1}H-NMR of compounds **22d** and **92-95** as 4-bond W-couplings were observed between equatorial protons. *meso*-Compounds such as 1,3,5-*O*-orthobenzoate triol **22d** generate strikingly simple ^{1}H-NMR spectra by symmetry. Once this symmetry is broken, for example by introduction of a protecting group on the 4-*O*, the 6 independent, although often overlapping, individual Ins-*H* resonances can be seen. The coupling occurring in these derivatives can be divided into three types, Figure 2.33.



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Figure 2.33: Observed couplings in the orthoester derivatives a) Coupling of two adjacent equatorial protons 2-4 Hz; b) Coupling of an adjacent equatorial and an axial proton 1-2 Hz; c) Coupling of two non-adjacent equatorial protons through the fixed ring structure (W-coupling) 1-2 Hz.

Interpretation of these spectra can be complex due to the amount of W-coupling and overlapping peaks. The expected resonance for each of the ring protons is detailed in Table 2.1.

Table 2.1: Expected splitting patterns of protons in ^{1}H-NMR for an orthoester derivative.

Proton	Orientation	Splitting by adjacent protons	Long range W-couplings	Observed peak
1-H	Equatorial	dd	2	dq
2-H	Axial	t	0	t
3-H	Equatorial	dd	2	dq
4-H	Equatorial	t	1	td
5-H	Equatorial	t	2	tt
6-H	Equatorial	t	1	td

The use of benzyl ethers as 2- and 6-*O* protecting groups also produces distinctive signals in the ^{1}H-NMR. Each benzyl-CH_2 resonates as a pair of roofed doublets between 4 and 5 ppm. These signals can overlap with the inositol protons, but it is generally possible to resolve the benzyl protons due to their striking splitting patterns and large (11-14 Hz) coupling constants.

In the ^{13}C-NMR of these derivatives, both the six Ins-*H* and two benzyl-CH_2 peaks fall in the 60-80 ppm region. However the peaks can be distinguished using DEPT-135.

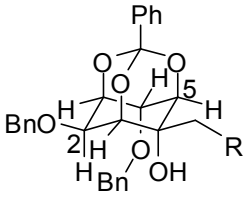
Each chapter of this thesis describes a new class of compound related to the synthesis of 4-*C*-alkyl analogues. The key transformations are each associated with characteristic changes in the NMR spectra, which will be introduced at the start of chapters three, four and five.

Chapter 3: Insertion of a 4-*C* alkyl group

Following the preparation of the inos-4-ose in my MChem project,[128] this chapter discusses the introduction of a 4-*C*-alkyl group with regeneration of the *myo*-inositol stereochemistry. In order to fully explore the scope of 4-*C*-alkyl protrusions available for RLE, the addition of primary, secondary and tertiary alkyl and aromatic nucleophiles was explored, either directly to, or separated from the inositol ring.

NMR played a key part in identifying the position and geometry of alkyl substitution on the inositol ring. The substitution is evident not only by the absence of one ^{1}H-NMR proton resonance, but also in the splitting patterns of adjacent protons, Table 3.1.

Table 3.1: Expected ^{1}H-NMR signals of protons in 4-*C*-alkyl orthobenzoate derivatives.



109, R = alkyl

Proton	Orientation	Splitting by adjacent protons	Long range W-couplings	Observed peak
1-H	Equatorial	dd	2	dq
2-H	Axial	t	0	t
3-H	Equatorial	d	2	q
4-H	-	-	-	-
5-H	Equatorial	d	2	dt
6-H	Equatorial	t	0	t

On substitution of the 4-*H* by an alkyl chain both the 3 and 5-*H* will couple through three bonds to only one adjacent proton; the 3-*H* is expected to resonate as an apparent quartet, and the 5-*H* as a double triplet. This substitution will also remove the W-couplings to the 6-*H*, which would now be expected to resonate as a triplet (*J* 2-4 Hz) due to its two equatorial neighbours. This triplet contrasts with the 2-*H* which, despite a gauche relationship to the adjacent 1- and 3-*H*, and therefore similar ~60 º dihedral angles to the 6-*H* and it's neighbours, displays a much narrower triplet (*J* 1-2 Hz). The substitution of one Ins-*H* would also be visible in the ^{13}C-NMR where the quaternary C would not be observed in the DEPT-135, leaving 5 C-H peaks in the 60-80 ppm region. In addition the peaks for the 4-*C*-alkyl substituent should be visible in both the ^{1}H and ^{13}C-NMR spectra.

3.1 Addition of alkyl Grignards to the inos-4-ose

2,6-*O*-Dibenzyl inos-4-ose 1,3,5-*O*-orthobenzoate **96** was prepared using the methodology described in section 2.5[128] (Figure 2.27). The experimental details for this sequence can be found in Appendix A.

Initial attempts to generate *exo*-methylene oxides were limited in their success. Both possible geometries of *exo*-methylene oxide were generated from oxidation of the *exo*-methylene using *m*CPBA, and low conversions were obtained on addition of sulfur ylides to the inososе, see section 2.5. Therefore it was chosen to confirm and hopefully extend the observations reported by Potter *et al.*[130] by direct addition of Grignard reagents to the inos-4-ose. Upon treatment of inos-4-ose **96** with methylmagnesium bromide at -78 °C selective equatorial addition of the methyl group was observed to afford 4-*C*-methyl orthobenzoate **115** with simultaneous regeneration of the *myo*-geometry 4-*O*H,[130] Figure 3.1.

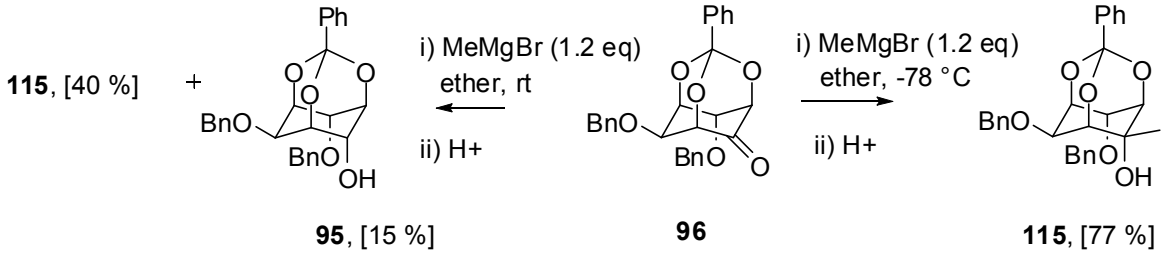


Figure 3.1: Addition of methyl Grignard to the inos-4-ose

The 4-*C* stereochemistry was confirmed in later steps by NOESY after unblocking of the orthoester (see section 4.1). Notably, addition of methylmagnesium bromide to inos-4-ose **96** at rt generates two distinct products; desired **115** in lower yield than at -78 °C, and the reduction product **95** (15 %) which was identical by ^{1}H-NMR to the isolated 4-*O*H prior to oxidation. It is peculiar that methylmagnesium bromide acts as a hydride donor. Although β-hydride delivery is well documented as a competing reaction for larger alkyl Grignard reagents with hindered ketones,[131-4] the absence of a β-proton in methylmagnesium bromide should prevent this reaction pathway. Despite this unusual outcome, both **115** and **95** are the result of equatorial delivery, of either methyl or hydride, to inos-4-ose **96**. This selectivity of addition may be attributed to a combination of steric hindrance of the bottom face of the carbonyl by the 6-*O*-benzyl ether and possible coordination of magnesium to the orthoester 3- and 5-*O*, Figure 3.2.

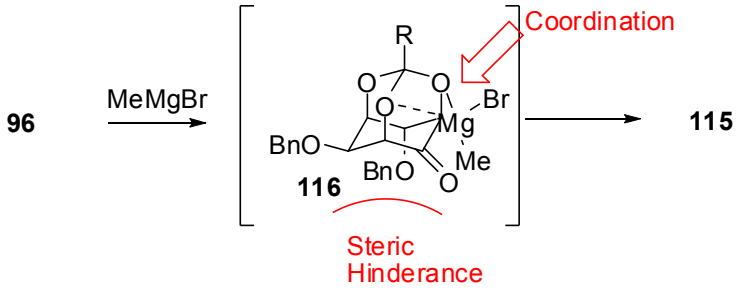


Figure 3.2: Addition of a methyl Grignard reagent is stereospecific

It was expected that larger 4-*C*-alkyl substituents could be introduced by addition of larger Grignard reagents to the inos-4-ose. However, addition of ethylmagnesium bromide to **96** at -78 °C generated only reduction product **95** in nearly quantitative yield due to β-hydride delivery, and none of the desired 4-*C*-ethyl product **117**, Figure 3.3.

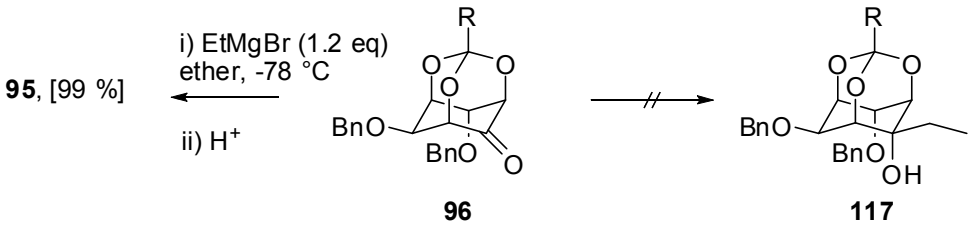


Figure 3.3: Reaction of ethyl Grignard with inos-4-ose **96**; reduction rather than addition.

The accepted mechanism for Grignard reagent addition to a ketone requires two equivalents of the alkyl Grignard.[132,133] Initial coordination to the ketone oxygen by the first equivalent is followed by alkyl delivery from the second. Were alkyl delivery to proceed from the coordinated Grignard reagent, this would require an unfavourable 4-membered ring intermediate, Figure 3.4a. However, β-Hydride reduction, can occur from the initially coordinated Grignard reagent via a 6-membered ring intermediate, Figure 3.4b, leading to increasing preference for the latter as the size of the Grignard reagent and steric hindrance of the ketone increase, preventing access of the second equivalent for alkyl delivery.[131]

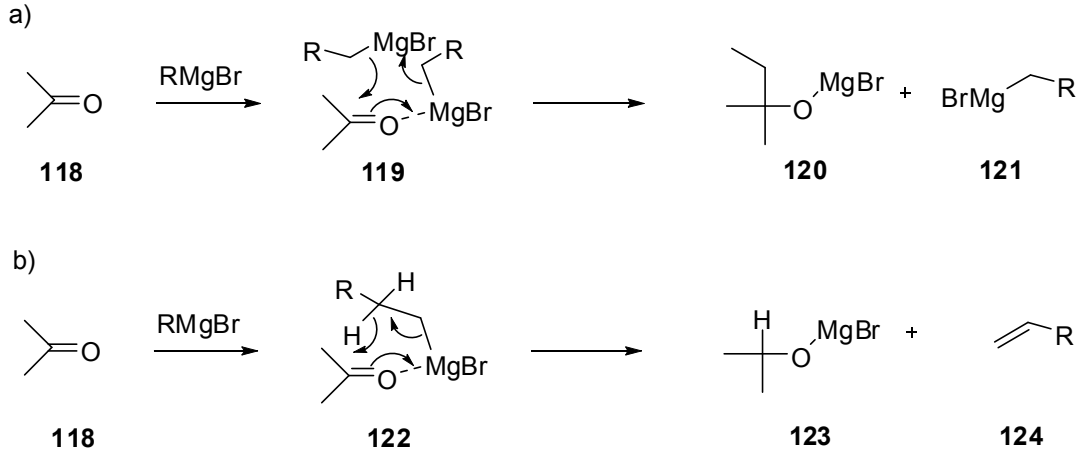


Figure 3.4: The mechanisms for the competing reactions of alkyl Grignards [adapted from ref. 132, 133]; a) mechanism of addition; b) mechanism of reduction

The preference for reduction with Grignard reagents larger than methylmagnesium bromide was confirmed by addition of *iso*-propyl Grignard reagent to inos-4-ose **96**, which also generated **95** as the only product. In an attempt to circumvent the generation of β-hydride reduction products, benzylmagnesium chloride was prepared. Upon addition to **96**, two products were isolated; 4-*C* benzyl **125** (10 %) and **95** (28 %) Figure 3.5. Again the presence of the reduction product **95** is hard to explain given the absence of β-hydrogens.

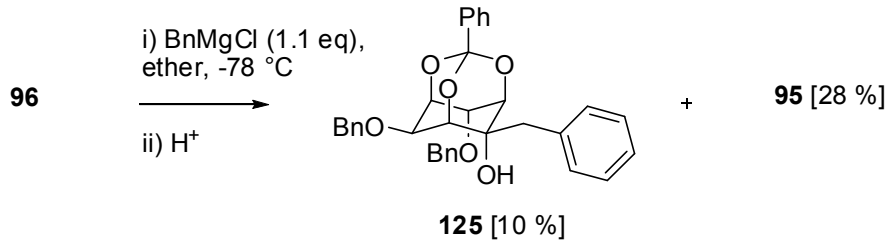


Figure 3.5: Removal of the β-hydrogens does not prevent elimination with benzyl magnesium chloride

Addition of Li^+, K^+ or Na^+ salts to dialkyl magnesium species has been reported to increase yields of addition in preference to reduction. However, little improvement in yield is observed for branched alkyls such as *tert*-butyl.[134] Similarly, addition of magnesium bromide prior to the alkyl Grignard reagent is reported to complex the ketone and, being a stronger Lewis acid than the Grignard reagent itself, promote addition reactions.[133] However, this method also has reduced success as the bulk of the Grignard reagent continues to increase. The importance of being able to introduce larger alkyl groups to create an orthogonal ligand for RLE has already been established. Therefore it was concluded that the use of alkyl Grignard reagents may not be the best means to achieve this goal.

3.2 Insertion of methylene to generate the *exo*-methylene oxide from the inos-4-ose

Very limited success was achieved with direct introduction of an alkyl group to the inososе, as with alkyl substituents larger than methyl, very little or none of the desired 4-*C*-alkyl product was isolated. However, of the reactions that were observed, delivery of the alkyl or indeed hydride, occurred stereospecifically from the top face of the inososе to regenerate the axial 4-*O*H of a *myo*-inositol orthoester. Furthermore, a disadvantage of introducing 4-*C*-alkyl groups using this method is that each new 4-*C*-substituent is required to be delivered in the same manner, and the *myo*-stereochemistry of each product must be verified in turn. It would be therefore preferable to set in place the desired stereochemistry, pre-determining the orientation of the alkyl group. This can be achieved by addition of methylene accross the inososе generating an *exo*-methylene oxide. It has already been postulated (section 2.3) that direct attachment of the 4-*C*-alkyl to the cyclohexane backbone may result in undesirable conformers in the final products. Conversion to the *exo*-methylene oxide simultaneously introduces the proposed methylene spacer between the alkyl group and cyclohexane backbone, and defines the 4-*C*-stereochemistry. Direct delivery of methylene to the inososе using sulfur ylide chemistry is reported to generate *exo*-methylene oxides.[96] It was hoped that, similarly to the Grignard reagents, steric bulk combined with available coordination to the orthoester would promote delivery of methylene to the top face of the inososе, generating *myo*-stereochemistry. From the generation of both *exo*-methylene oxides on *m*CPBA oxidation of the *exo*-methylene,[128] it was known that desired *exo*-methylene oxide **98**'s protons resonate as paired doublets in ^{1}H-NMR at 2.94 and 2.93ppm.

myo-Inositol *exo*-methylene oxide **98** was prepared from **96** using dimethylsulfoxonium methylide, generated by treatment of trimethyloxosulfonium iodide in DMSO-THF with sodium hydride at 0 ºC,[135] Figure 3.6.



98 [9 %] **108** [24 %] **98** [89 %]

Figure 3.6: Conversion of the inos-4-ose to the *exo*-methylene oxide

Initial test reactions had been carried out using the method of Corey and Chaykovski[135] in DMSO at 50 ºC and produced a mixture of the oxirane **98** and oxetane **108** as a result of a second methylene insertion into the oxirane.[128] By reducing the temperature to 0 ºC for preparation of the ylide, followed by addition of the inos-4-ose as a solution in THF at rt, it was possible to prepare the *exo*-methylene oxide **98** in high yield, with no evidence of the oxetane. In all cases, including

the initial reactions at elevated temperatures, no evidence of axial methylene addition was observed.

exo-Methylene oxide **98** was reduced with excess $LiAlH_4$ in THF to yield the equatorial 4-*C*-methyl product **115**, Figure 3.7.

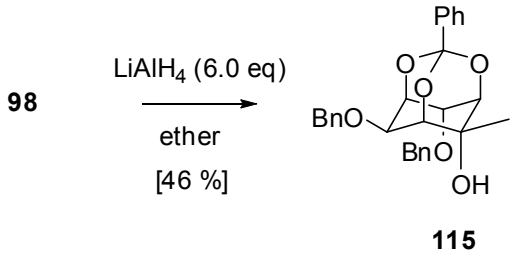


Figure 3.7: Reduction of the *exo*-methylene oxide to the 4-*C*-methyl confirms the 4-*C*-stereochemistry

Only the product of nucleophilic attack at the unsubstituted end of the epoxide was observed, controlled by steric hindrance. Comparison to the product from addition of methyl Grignard to the inosose verified that the desired *myo*-inositol stereochemistry *exo*-methylene oxide had been prepared.

3.3 Addition of alkyl Grignards to the *exo*-methylene oxide

It was expected that opening the *exo*-methylene oxide with an alkyl Grignard reagent would generate 4-*C*-alkyl products including a CH_2 spacer, with pre-determined stereochemistry. As for the inosose, alkyl anion addition to the *exo*-methylene oxide will be in competition with β-hydride reduction. However it was anticipated that this competition would be reduced due to the less crowded environment around the unsubstituted carbon of the epoxide.

Surprisingly, addition of methyl Grignard to **98** generated 4-*C*-bromomethyl **126**, by nucleophilic halide attack on the epoxide, Figure 3.8.

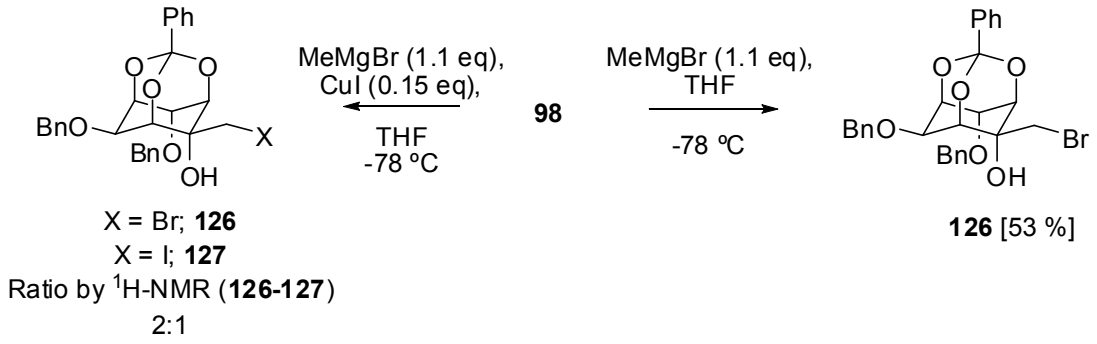
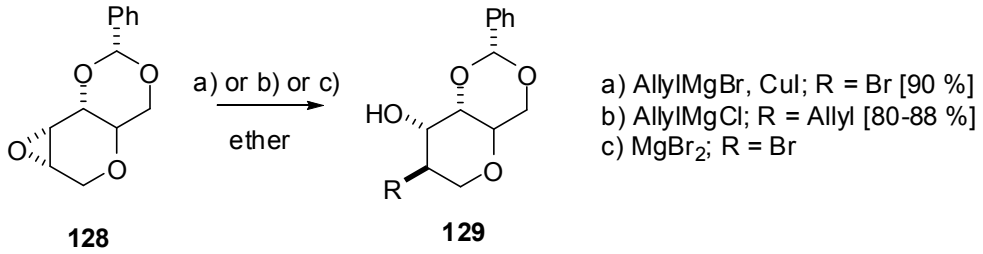


Figure 3.8: Unexpected halide delivery to open the *exo*-methylene oxide

From this unexpected product, an S_N2 displacement was attempted to generate the desired 4-*C*-alkyl product. However, addition of excess methyl Grignard to **126** did not effect any further

reaction. It was postulated that removal of the bromine from this reaction would prevent formation of **126**. Therefore precipitation of magnesium bromide in dioxane, using the Schlenk equilibrium to generate dialkyl magnesium, was attempted.[132] However, on addition of the dialkyl magnesium to **98**, no reaction was observed and only starting material recovered.

Attempts were made to generate a softer nucleophile by *trans*-metallation of methyl Grignard with copper iodide,[136] however addition of this organocuprate to the *exo*-methylene oxide generated a mixture of the 4-*C*-bromomethyl, **126**, and 4-*C*-iodomethyl, **127**, Figure 3.8. These two compounds were identified by the presence of a pair of roofed doublets (δ_H 3.80, 3.92 and 3.90, 4.05ppm) in the ^{1}H-NMR spectrum, two quaternary centres (δ_C 68.9 and 69.8ppm) and two methylenes (δ_C 38.8 and 15.1ppm) in the ^{13}C-NMR. The generation of a similar brominated compound was observed by Brockway *et al.*[137] during the reaction of an epoxide with allylmagnesium bromide under copper catalysis, Figure 3.9.



128 **129**

Figure 3.9: Unexpected opening of epoxide with bromide, adapted from ref. 137

Unlike the mixture of bromo- and iodomethyl compounds observed upon opening of **98**, they did not observe any iodomethyl derivative. They suggest a Lewis acid catalysed scission of the oxirane ring, having observed formation of the same brominated compound upon addition of $MgBr_2$ to their oxirane.[137] The reaction of **128** with allylmagnesium chloride generated their desired alkyl product in 80-88 % yield, suggesting that the generation of methylhalide species may be specific to bromo Grignard reagents. The increased reactivity of **98** towards bromide or iodide may be a result of increased strain on the epoxide induced by the rigid orthoester inositol framework.

3.4 Addition of alkyl lithiums to the *exo*-methylene oxide

One of the main obstacles thus far, had been the lack of successful introduction of any 4-*C*-alkyl substituent larger than methyl, which was a key part of the synthetic strategy. The generation of the 4-*C*-bromomethyl orthobenzoate **126** during treatment of the *exo*-methylene epoxide **98** with alkyl Grignard suggested a halide-free alkylating reagent, such as an alkyl lithium may be more successful. Due to the instability of alkyl lithium solutions during storage, the concentration of each alkyl lithium solution was titrated immediately prior to use using salicylaldehyde phenylhydrazone as an indicator, prepared according to the method described by Love and Jones.[138]

The titration end point is recognised as a colour change from yellow to red, which indicates the start of the second deprotonation, Figure 3.10. With darker alkyl lithiums such as PhLi, it was important that the solution was sufficiently dilute (50-80 mg indicator in 5 mL THF for a 1 M solution of Grignard reagent) in order that the end point of the titration could be seen above the colour of the reagent.

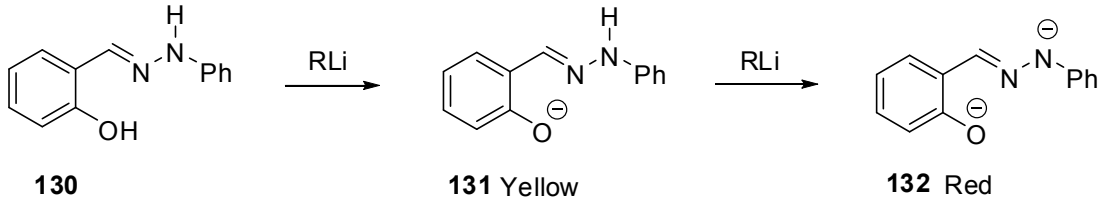


Figure 3.10: Salicylaldehyde phenylhydrazone as an indicator for titration of organometallic reagents

In order to obviate the possibility of competing β-hydride insertion to the *exo*-methylene oxide, it was decided to first attempt treatment of **98** with phenyl lithium. Careful addition of 2 eq. PhLi slowly converted the starting material to a single spot by TLC. The desired 4-*C*-benzyl, **125**, was recovered following chromatography but despite multiple attempts, yields were very variable and the maximum yield achieved was 66%, Figure 3.11.

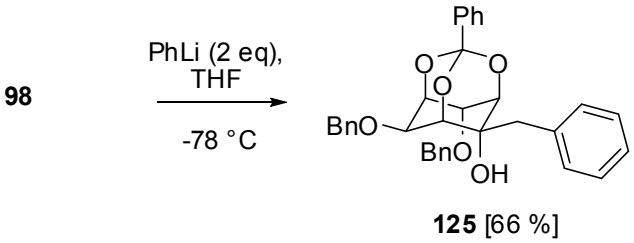


Figure 3.11: Addition of phenyl lithium to the *exo*-methylene oxide

In the hope of expanding the range of 4-*C*-alkyl substituted products, addition of other alkyl lithiums to **98** was attempted. Treatment of **98** with *n*-butyl lithium afforded 4-*C*-pentyl **133** but only in 18 % yield, Figure 3.12.

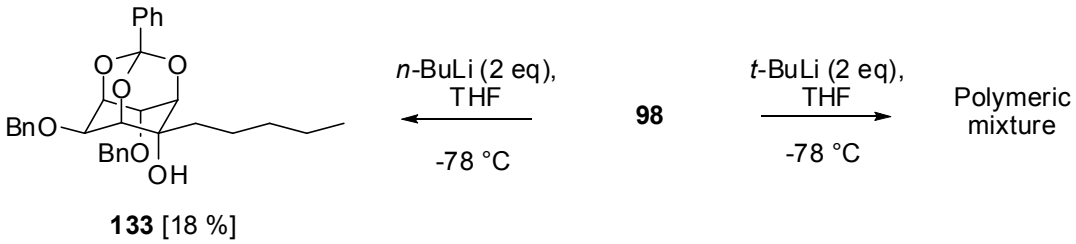


Figure 3.12: Addition of *n*-butyl lithium and *t*-butyl lithium to the *exo*-methylene oxide

Addition of bulky *tert*-butyl lithium to **98** generated none of the desired 4-*C*-alkyl product. During these reactions, streaking was observed on the TLC plate, and ^{1}H-NMR of the crude

material was broad in the inositol region. This suggested that the poor recovery of these species, without the generation of any other distinct products may be a result of competing polymerisation. This could occur due to deprotonation on the phenyl rings of the benzyl ethers or orthobenzoate of **98**, which would then go on to react with further equivalents of **98**, generating oligomers. MS of the crude material from the reaction of **98** with *tert*-butyl lithium suggested that polymerisation had occurred as *m/z* peaks were observed at ≈ $[2M+H]^+$ and $[4M+H]^+$, corresponding to side reactions such as those shown in Figure 3.13.

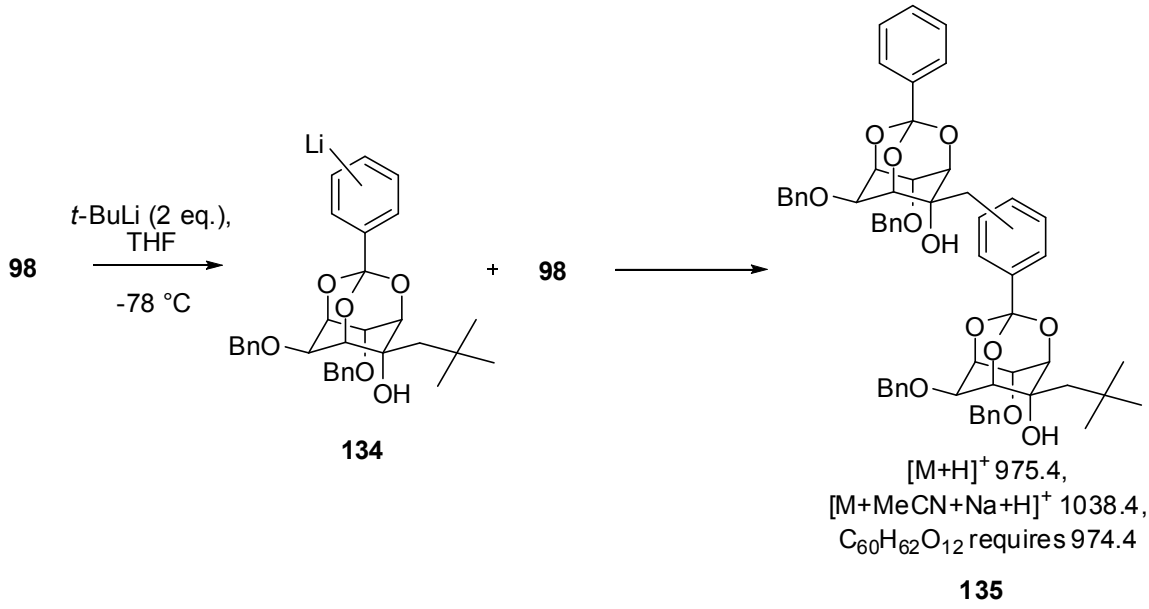


Figure 3.13: Polymerisation of **98** on addition of *tert*-BuLi

Unlike with the Grignard reagents, no products from β-hydride delivery were observed. An increased tendency for polymerisation down the sequence PhLi → *n*-BuLi → *t*-BuLi may be reflected by the isolated yields of the desired products. This trend correlates with increasing carbanion basicity, leading to an increased degree of polymerisation due to competing deprotonation overtaking epoxide opening from nucleophilic attack.

3.5 Addition of alkyl lithium cuprates to the *exo*-methylene oxide

It was hoped that generating a less basic nucleophile would reduce deprotonation of the aryl rings and therefore the competing polymerisation. Higher order mixed cuprates of the form $R_2Cu(CN)Li_2$, generated by addition of an alkyl lithium to copper cyanide in a 2:1 ratio, are reportedly among the mildest and most efficient method for generating C-C bonds.[139] They have been shown to effect nucleophilic opening of epoxides in high yield.[139] It was postulated that the alkyl lithium cuprates might be less basic nucleophiles than the corresponding alkyl lithiums. The alkyl lithium cuprates were prepared by drop-wise addition of alkyl lithium to a suspension of copper (I) cyanide in dry ether, at -78 ºC. It was essential that the copper cyanide was dried

immediately prior to use by repeated evaporation from dry toluene as the presence of moisture or allowing the temperature to exceed -60 ºC quickly destroyed the alkyl lithium cuprate, and could be observed by the formation of a black precipitate presumably CuO.

3.5.1 Addition of primary 4-*C*-alkyl chains

The conditions were optimised for the addition of *n*-butyl lithium cuprate to **98**. Initial reactions carried out in THF achieved a maximum yield of 48 % (Table 3.2, A and B). It is suggested that this is the result of the coordination of this solvent to the alkyl lithium causing aggregation, which may destabilise the alkyl lithium cuprate complex.[140]

Table 3.2: Optimising the conditions for the reaction of **98** with *n*-butyl lithium cuprate

Entry	Reaction Time (h)	Reaction Temp. (ºC)	Eq. CuCN	Eq. *n*-BuLi	Solvent	Starting Mat., 98 (%)	Product, 133 (%)
A	2	-20	1.46	2.67	THF	34	48
B	2	-78	1.46	2.67	THF	23	35
C	2	-78	1.46	2.67	Ether	22	67
D	2	-78	4.00	8.00	Ether	12	68
E	4	-78	4.00	8.00	Ether	0	70

It was necessary to use a large excess of alkyl lithium cuprate; typically 8 equivalents alkyl lithium and 4 equivalents CuCN per equivalent of *exo*-methylene oxide (Table 3.2, E) to ensure that the reaction went to completion. It is not known why such a high concentration of reagent was required. However it is possible that the inositol ring oxygens may coordinate to the alkyl lithium cuprate complex causing it to partially decompose, in a similar manner to the known coordination of solvents such as THF.[140]

Addition of primary alkyl lithium cuprates to *exo*-methylene oxide **98** using the best conditions now generated the 4-*C*-ethyl- and 4-*C*-pentyl-*myo*-inositol orthobenzoates in high yields (92 and 90 % respectively), Figure 3.14.

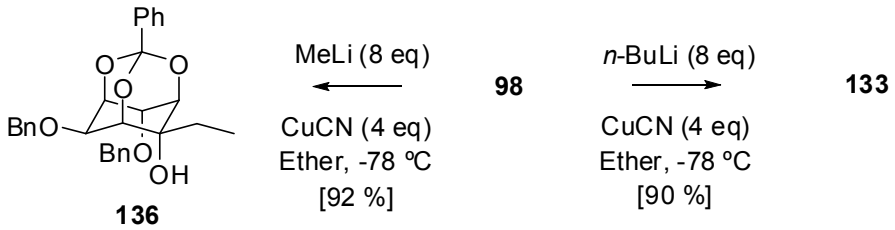


Figure 3.14: Preparation of 4-*C*-ethyl and 4-*C*-pentyl substituents by addition of alkyl lithium cuprates to *exo*-methylene oxide **98**

The increase in yield of **133** (from 18 % with alkyl lithium to 90 % with alkyl lithium cuprates) suggested that these reagents were considerably less basic, and that competing polymerisation had been suppressed.

3.5.2 Addition of secondary 4-*C*-alkyl chains

Addition of *iso*-propyl lithium cuprate to the *exo*-methylene oxide generated two distinct products. Desired 4-*C*-*iso*-butyl **137** was isolated in only 26 % yield, Figure 3.15.

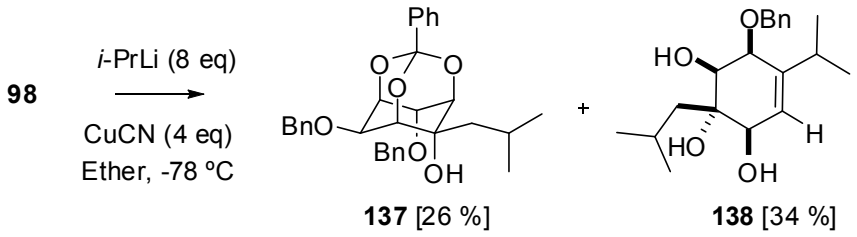


Figure 3.15: Treatment of *exo*-methylene oxide **98** with *iso*-propyl lithium cuprate generates two products

A second compound was crystallised from the crude material with an unexpected ^{1}H-NMR resonance at δ 5.74 (1H, d, *J* 5.4) suggesting elimination had occurred to generate an alkenyl proton. Also present were two sets of *iso*-propyl methyl doublets (δ_H 1.12, 1.08, 1.06, 1.02 ppm) suggesting a second addition of the *iso*-propyl nucleophile. A crystal structure, obtained despite twinned crystals, established the identity of this side product as conduritol derivative **138**, Figure 3.16.

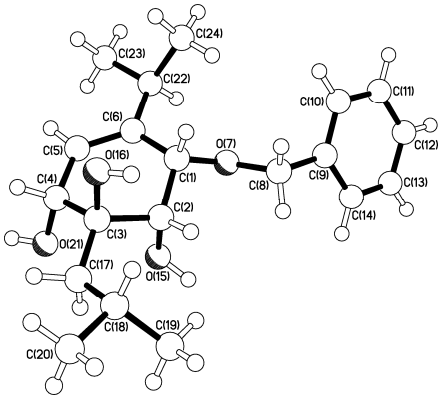


Figure 3.16: Structure of unexpected conduritol derivative **138** (Systematic name: 6-benzyloxy-2-isobutyl-5-isopropyl-cyclohex-4-ene-1,2,3-triol).

The mechanism of this transformation is not clear. However this side product was recovered in equal or higher yield than the desired 4-*C*-*iso*-butyl derivative during each attempt at this transformation. In order to establish whether the reaction was independent of epoxide opening, 4-*C*-methyl orthobenzoate **115** was treated with *iso*-propyl lithium cuprate, in the same manner as

exo-methylene oxide **98**. The corresponding product, **139**, was generated in 30 % yield (with the remainder being recovered as starting material), Figure 3.17.

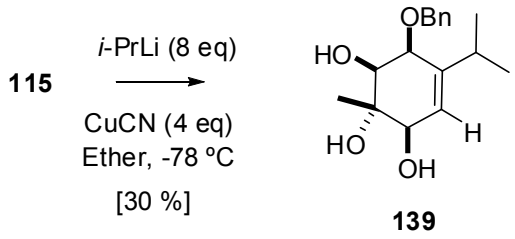


Figure 3.17: The corresponding conduritol derivative was recovered upon treatment of **115** with *iso*-propyl lihtium cuprate, systematic name: 6-benzyloxy-2-isobutyl-5-methyl-cyclohex-4-ene-1,2,3-triol

This confirms that the observed cleavage of the orthobenzoate and elimination of the 6-*O*-benzyl are independent of epoxide opening. A mechanism has been proposed by Crandall and Lin[141] to rationalise side products which they observed during epoxide opening with alkyl lithiums, proceeding via a carbene intermediate. The following mechanism is therefore suggested for generation of conduritol derivatives **138** and **139**, Figure 3.18.

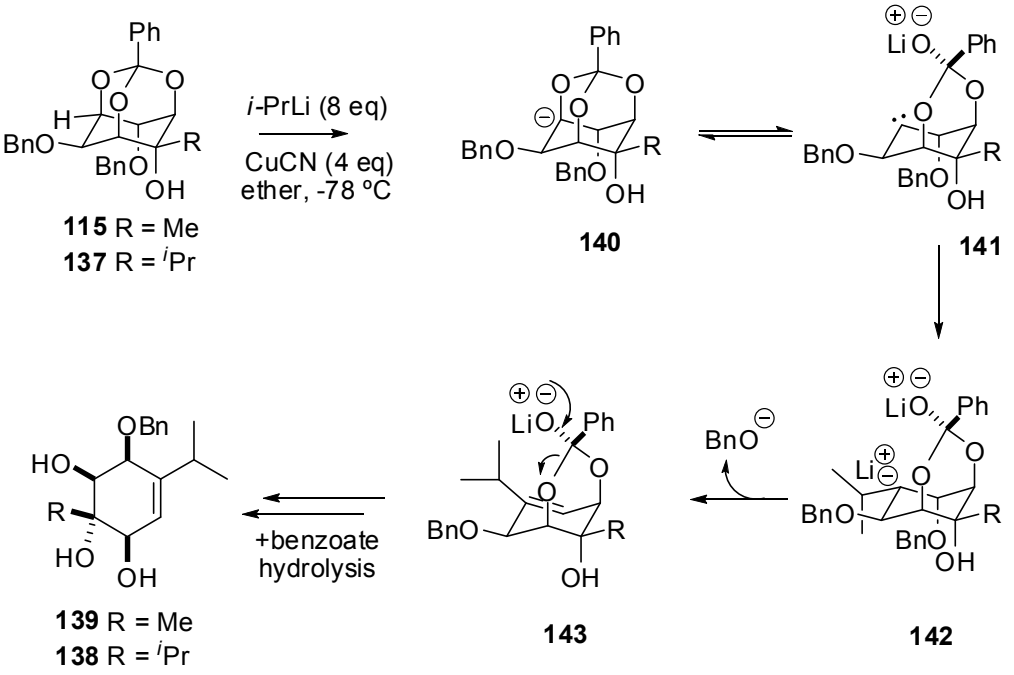


Figure 3.18: Suggested mechanism for the generation of conduritol side products

Following the isolation of these unusual compounds, the ^{1}H-NMR spectra of all alkylation reaction were examined for evidence of allylic protons which would indicate the presence of similar species. However, no such peaks were found, so it would appear that this side product is specific to the *iso*-propyl lithium cuprate.

3.5.3 Addition of tertiary lithium cuprates to the *exo*-methylene oxide

Upon preparation of *tert*-butyl lithium cuprate solution, particulate material began to form and the solution became brown and discoloured. However, the *exo*-methylene oxide was completely consumed within the 4 h reaction time. Two products were recovered; desired 4-*C*-neopentyl **144**, and the result of β-hydride opening of the *exo*-methylene, 4-*C*-methyl orthobenzoate **115**, Figure 3.19.

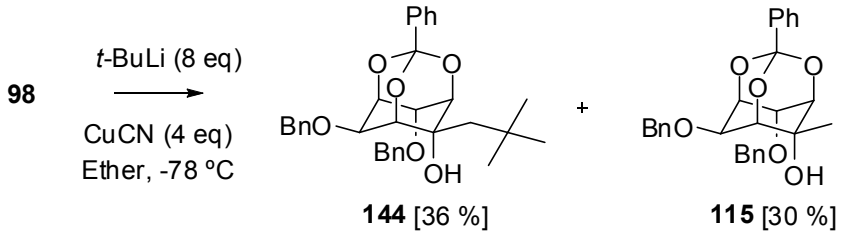


Figure 3.19: The addition of *tert*-butyl lithium cuprate to *exo*-methylene oxide **98** generates two products

This was the first observation of β-hydride reduction products during the nucleophilic opening of epoxide **98**. In order to try and increase the preference for desired **144** over **115**, a series of experiments were carried out, Table 3.3.

Table 3.3: Experiments attempting to improve the ratio of **144**:**115**

Entry	Reaction Time (h)	Reaction Temp. (ºC)	Eq. CuCN	Eq. *t*-BuLi	Eq. BF_3	Ratio of Products (^{1}H-NMR of crude material)		
						98	144	115
A	4.0	-78	4	8	-	0.0	2.0	3.0
B	4.0	-78	4	8	-	0.0	1.0	2.0
C	1.5	-78	3	6	-	5.0	6.0	5.6
D	4.0	-78	6	6	-	1.0	2.6	4.0
E	4.0	-78	3	6	1.0	3.0	7.0	3.6
F	4.0	-78 → -50	4	8	1.2	1.0	7.0	3.6

It is apparent that the temperatures and reaction times originally used (-78 ºC, 4 h, Table 3.3, A and B) are required to allow completion of the reaction. Shorter reaction times (entry C) contained large amounts of unreacted *exo*-methylene oxide **98**, without any evidence of improvement in the ratio of products. It was hoped that different stoichiometries of copper might influence the formation of one product over the other, but this also had little impact on the product ratio, entry D. The addition of a Lewis acid such as BF_3, is reported to enhance the rate of reaction between an epoxide and alkyl lithium cuprate for less reactive epoxides,[142] by coordinating to the epoxide oxygen, promoting nucleophilic attack on the adjacent carbon centre. Alexis *et al.*[143] report a 78 % yield for the opening of cyclohexene oxide with *tert*-butyl lithium cuprate and

$BF_3.Et_2O$ in ether. It was hoped that the addition of BF_3 would increase the rate of alkyl, rather than β-hydride, delivery to **98** (entries E and F). In both cases, there was still unreacted epoxide, perhaps suggesting competing reagent destruction by the Lewis acid. However, the ratio of products obtained was improved, with almost a 2:1 preference for desired **144**.

3.5.4 Addition of aryl lithium cuprates to the *exo*-methylene oxide

Addition of phenyl lithium cuprate to *exo*-methylene oxide **98** afforded 4-*C*-benzyl, **125** in 90 % yield (*cf.* 66 % for phenyl lithium alone, section 3.4), Figure 3.20.

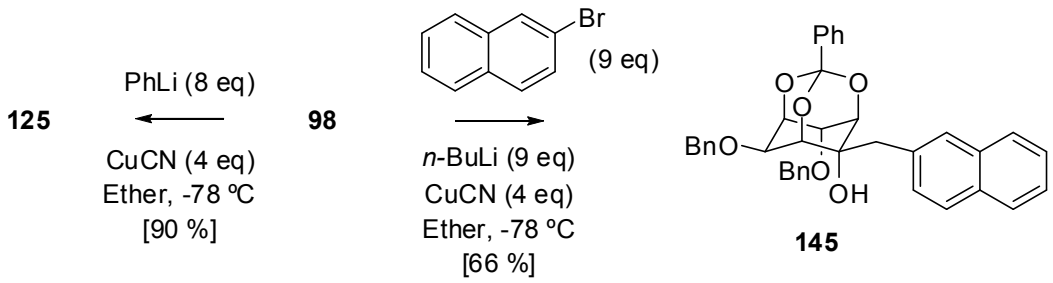


Figure 3.20: Addition of phenyl lithium cuprate and 2-naphthyl lithium cuprate to *exo*-methylene oxide **98**

Larger, more diverse aryl lithiums are not available as commercially prepared solutions. Lithium-naphthyl and β-lithium-styrene were prepared by lithium halogen exchange from their respective bromides using *n*-butyl lithium.[144] The small scale of preparation and thermal instability of these compounds meant they could not be easily titrated using the methods described for the commercially available solutions. Therefore, the exact stoichiometry of the aryl lithium in the reaction mixture is unknown.

2-Bromo-naphthalene was treated with *n*-butyl lithium at rt to prepare 2-lithium-naphthylene.[144] The solution was then cooled to -78 ºC before addition to the ethereal copper cyanide suspension at -78 ºC to generate the cuprate. Addition of lithium naphthyl cuprate to *exo*-methylene oxide **98** produced the desired 4-*C*-(2-naphthyl)methyl product (66 %), with the remainder being recovered starting material, Figure 3.20.

β-Bromo-styrene was treated with *n*-butyl lithium to generate β-lithium styrene. Preparation of the aryl lithium was carried out at -20 ºC to prevent the reaction solution from solidifying. The use of higher temperatures resulted in decomposition of the aryl lithium. The solution was then cooled to -78 ºC before addition to the ethereal copper cyanide solution. A single product was observed on addition of the cuprate to **98** and isolated by chromatography. Absence of the expected alkenyl peaks at δ 5-6 ppm or any splitting of the characteristic 4-*C*-CH_2 doublets at 4.03 and 3.89 ppm was not encouraging. Comparison with earlier ^{1}H-NMR spectra from the reaction with methyl Grignard and *exo*-methylene oxide **98** and HRMS confirmed that the single product formed was 4-*C*-bromomethyl, **126**, Figure 3.21.

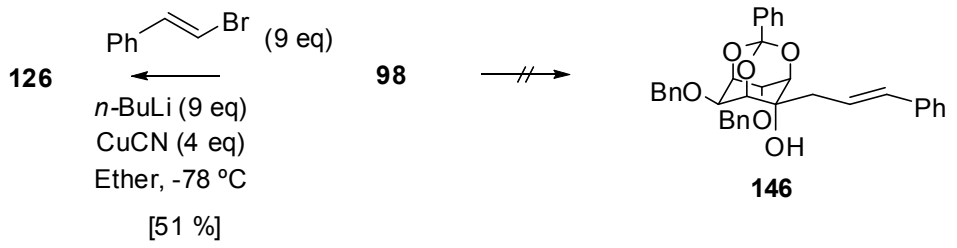


Figure 3.21: Preparation of β-lithium styrene and addition to *exo*-methylene oxide **98**

The preparation of 4-*C*-(β-methyl)styrene was expected to provide a route to 4-*C*-aryl substituents with the phenyl ring at an increased distance from the inositol cyclohexane backbone. Upon later removal of the 2,6-*O*-dibenzyl ethers, the double bond would be reduced to generate a 4-*C*-(3-phenylpropyl) substituent. It was also hoped that this group may provide a simple method of generating a wide variety of 4-*C* substituents. For example, by dihydroxylation of the double bond followed by removal of the benzyl fragment by osmylation would generate a 4-*C*-acetaldehyde which could be elaborated to generate further products. Further oxidation to the 4-*C*-ethanoic acid, for example, would generate a ligand which may be suitable for use in ion-pair exchange RLE, Figure 3.22.

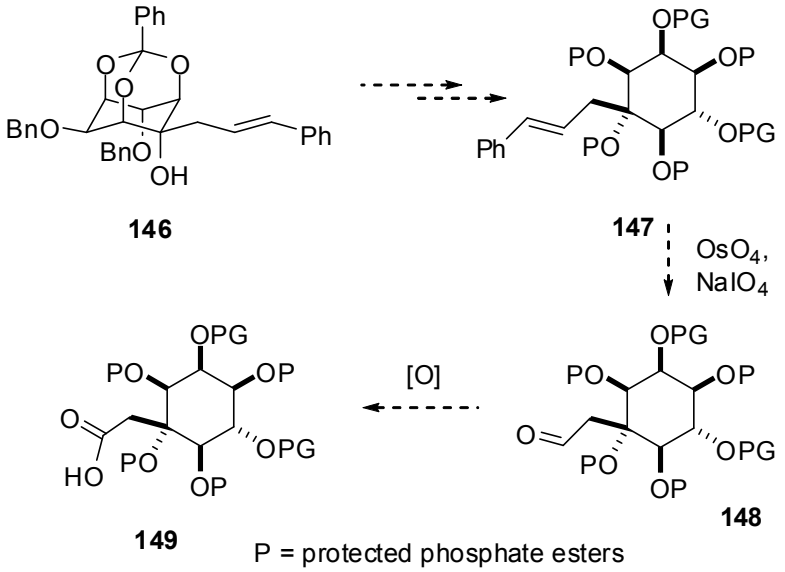


Figure 3.22: Manipulation of 4-*C*-(3-phenylprop-2-enyl) **146** has the potential to generate ligands suitable for ion-pair exchange RLE

In a parallel project in this laboratory, the related conversion of 2-*O*-allyl nonabenzyl Ins(1,3,4,5)P_4 to the corresponding 2-*O*-acetic acid has already been optimised. In future it may be easier to use acetylide or phenyl acetylide anions to open the epoxide since they can be easily generated without the presence of halide salts.

3.6 Concluding Remarks

The scope of alkyl and aryl introduction for steric complementation ligand engineering has been broadly investigated. The addition of alkyl lithium cuprates to the *exo*-methylene oxide proved by far the most reliable and generalisable method for introducing a 4-*C*-alkyl group. The 4-*C*-methyl (from reduction of the *exo*-methylene oxide), 4-*C*-ethyl, 4-*C*-pentyl, 4-*C*-*iso*-butyl, 4-*C*-neopentyl, 4-*C*-benzyl and 4-*C*-(2-napthyl)methyl have all been successfully prepared, and could be synthesised on a large scale. The low yield for more hindered alkyl nucleophiles (*iso*-propyl lithium and *tert*-butyl lithium) has been explained by the characterisation of reaction side products. In order to access ion-pair exchange derivatives the preparation of β-lithium styrene would require modification, for example by removal of the lithium bromide prior to addition to the *exo*-methylene oxide. There are a number of other routes which could be applied to introduce a similarly oxidisable group in this position, thus generating a more diverse library of ligands. However this was beyond the scope of this project.

Chapter 4: Manipulation of the orthoester to generate 4-*C*-alkyl *myo*-inositol triols and tetrols

Following the introduction of a 4-*C*-alkyl group, this chapter explores the removal of temporary protecting groups to generate free hydroxyls at the sites of phosphorylation. It was hoped that both the required IP_3 and IP_4 precursors could be accessed by manipulation of the orthobenzoate. Section 4.1 explores the acidic hydrolysis of 4-*C*-alkyl-*myo*-inositol 1,3,5-*O*-orthobenzoates to generate Ins(1,3,4,5)P_4 precursors, the 4-*C*-alkyl-2,6-*O*-dibenzyl-*myo*-inositol tetrols. Section 4.2 reports the preparation of 4-*C*-alkyl-*myo*-inositol orthoformates as an alternative strategy for obtaining these precursors. Section 4.3 addresses 2,4,6-*O*-tribenzyl-*myo*-inositol orthobenzoate as a model system for studying reduction of the orthobenzoate using DIBAL-H, followed by a study of the influence of different protecting groups on the regioselectivity of this deprotection. In sections 4.4 and 4.5, these results are applied to the 4-*C*-alkyl-*myo*-inositol 1,3,5-*O*-orthobenzoates to generate Ins(1,4,5)P_3 precursors, the 4-*C*-alkyl-2,3,6-*O*-tribenzyl-*myo*-inositol triols. The side products of DIBAL-H reduction, the 4-*C*-alkyl benzylidene acetals, are employed in section 4.6 as an alternative route for generating Ins(1,3,4,5)P_4 precursors.

Once again, NMR spectra of these transformations convey crucial information about the enviroment of the inositol ring protons. Removal of the 1,3,5-*O*-orthoester will allow the *myo*-inostiol ring to adopt its natural chair conformation once more, as the rigidly constrained adamantane-like structure is relieved. As a result, the ^{1}H-NMR of these species will no longer display 4-bond W-coupling. Returning to this configuration with a 4-*C*-alkyl substituent, there are now four axial and one equatorial protons, giving rise to two possible types of coupling, Figure 4.1

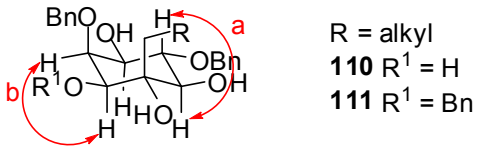


Figure 4.1: Observed couplings after removal of the orthoester; a) Anti-periplanar coupling between adjacent axial protons (dihedral angle 180 º) 7-10 Hz; b) Coupling between adjacent axial and equatorial protons (dihedral angle 60 º) 1-4 Hz.

The combination of multiplicity indicating the number of adjacent neighbours and splitting patterns indicating orientation of neighbours, alongside COSY spectroscopy, should permit identification of the regiochemistry resulting from deprotection of the orthoester. The stereochemistry of alkyl introduction should be confirmed at this stage by NOESY interaction between the 4-*C*-alkyl and 6-*H* which have a 1,3-diaxial relationship.

Table 4.1: Splitting patterns of protons in ^{1}H-NMR for a 4-*C*-alkyl triols and tetrols.

Proton	Orientation	Splitting by adjacent protons
1-H	Axial	dd
2-H	Equatorial	t
3-H	Axial	d
4-H	-	-
5-H	Axial	d
6-H	Axial	d

Partial deprotection of the orthoester, for example using DIBAL-H leading to an acetal will also demonstrate loss of the highly constrained structure which led to W-coupling in the ^{1}H-NMR spectrum. However in this case the ring will not be free to return to a chair conformation and it might be expected that more unusual coupling patterns may be seen, due to formation of boat-like structures to ease steric congestion. The regiochemistry of partial opening of the orthoester can be interpreted by the coupling of the protons in ^{1}H-NMR, with the aid of COSY, NOSEY, and D_2O exchange experiments, to identify the location of the free hydroxyl. Complete reduction of the orthoester with DIBAL-H to generate a benzyl ether for IP_3 synthesis can be observed in the 5 ppm region of the spectra, where an additional two doublets should appear corresponding to the benzyl-CH_2.

^{13}C-NMR of the inositol peaks should not change significantly in this step. However, deprotection of the orthoester can be observed by loss of the bridgehead quaternary-C at 100-110 ppm, and the appearance of an acetal-CH (at 93 ppm), benzyl-CH_2 from DIBAL-H reduction (60-80 ppm) or quaternary benzoate-C=O from acidic hydrolysis (165-170 ppm).

4.1 Acidic hydrolysis of the orthobenzoate

The acidic hydrolysis of 2,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate to a mixture of the 1- and 3-*O*-benzoate esters is already reported.[73] It was hoped that the 4-*C*-alkyl 2,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoates would generate the corresponding 1-, 3-, and possibly 5-*O*-benzoate esters.

To accomplish acidic hydrolysis of 4-*C*-methyl orthobenzoate, **115**, required concentrated HCl-methanol (1:2 v/v) at reflux, generating a mixture of benzoate esters Figure 4.2.

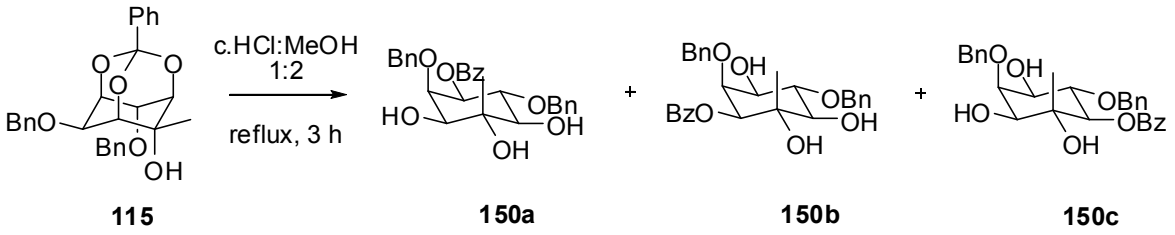


Figure 4.2: Acidic hydrolysis of 4-*C*-methyl orthobenzoate

Potter's conditions[73] for the unsubstituted 2,6-*O*-dibenzyl-*myo*-inositol orthobenzoate are 1.0 M HCl-ethanol (1:2) at reflux, for 5 h. No reaction was observed under these conditions with the 4-*C*-methyl orthobenzoate **115**, nor under the reported conditions for acidic hydrolysis of the orthoformate, TFA-H_2O (4:1, rt, 24 h).[100] Unlike acidic hydrolysis of unsubstituted orthobenzoates, which were reported to generate only the 1- and 3-*O* benzoate esters, hydrolysis of 4-*C*-methyl orthobenzoate generated all three benzoate esters.

The crude mixture of 4-*C*-methyl benzoate esters could be separated by flash chromatography. The 1-*O*-benzoate ester **150a** was isolated in 29 % yield and fully characterised. The 1-*O* benzoate esters are suitable precursors for PIP_3 lipid synthesis, allowing the introduction of the phosphatidate phosphodiester at the 1-*O* as has been demonstrated by Potter *et al.*[145]

For the purposes of 4-*C*-methyl Ins(1,3,4,5)P_4 synthesis, the crude mixture of benzoate esters was treated with NaOMe in methanol to generate the desired 2,6-*O*-dibenzyl tetrol **151a**, Figure 4.3

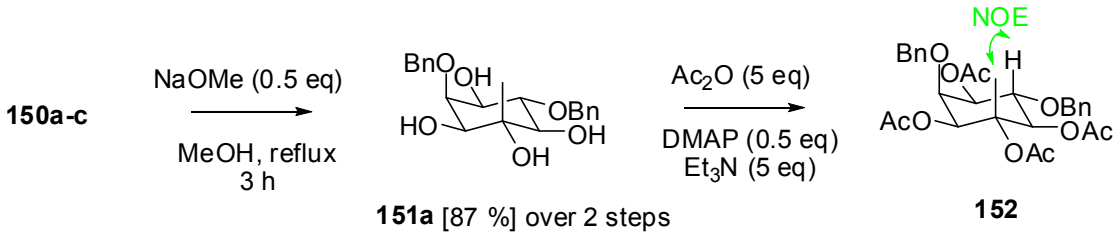


Figure 4.3: Transesterification of benzoate esters with NaOMe in MeOH generates 4-*C*-methyl tetrol, **151a**

The stereochemistry of 4-*C*-methyl addition was confirmed by acetylation of the four free hydroxyls and subsequent observation of an NOESY interaction between the 6-*H* and 4-*C*-methyl protons. Since the stereochemistry was fixed at the point of epoxide formation by the addition of methylene across the inos-4-ose, all other alkyl substituents which are derived from this same intermediate must therefore have the same geometry. For 4-*C*-alkyl groups larger than methyl, refluxing propanol-concentrated HCl (2:1 v/v) for 3 h was required to initiate transesterification. In all cases, a mixture of fluxional compounds was obtained. The mass was confirmed by HRMS and two spots were visible on the TLC plate, with lower R_f than the starting material. The crude

mixtures of benzoate esters were treated with NaOMe in methanol, as for the 4-*C*-methyl derivative, to generate a series of 1,3,4,5-tetrols, Figure 4.4.

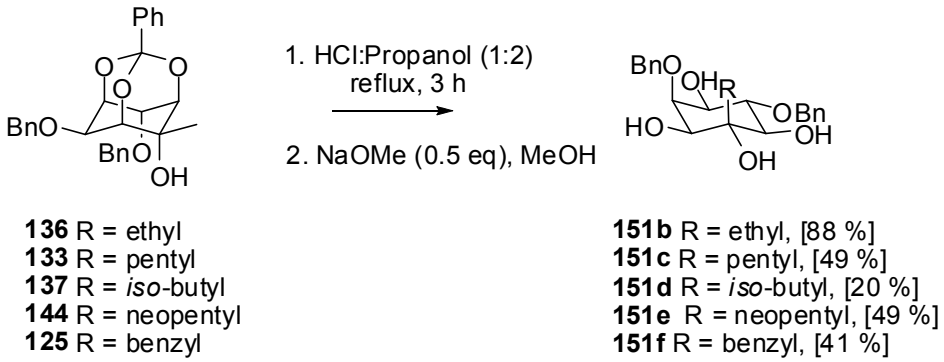


Figure 4.4: Acidic hydrolysis of 4-*C*-alkyl orthobenzoates followed by NaOMe in MeOH generates tetrols

The yields from the two-step orthobenzoate deprotection were noticably lower with larger 4-*C*-substituents. While the mass recovery was consistent for the sequence of reactions, after short column chromatography to purify the tetrol, there was a considerable loss of material. Identification of the problem was hampered by the fluxional nature of both the mixed benzoate esters and the 4-*C*-alkyl tetrols, making it difficult to observe or identify impurities. Acetylation of the crude 4-*C*-benzyl tetrol generated a number of products, suggesting that deprotection of the orthoester was not proceeding cleanly. A white solid was often seen to form in chloroform solutions after prolonged standing. Consequently crystallisation of crude 4-*C*-benzyl tetrol **151f** from CH_2Cl_2 was attempted from which a powdery white substance was isolated. This was demonstrated by HRMS to be 4-*C*-benzyl pentols **153** and **154** [HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 383.1456, $C_{20}H_{24}O_6Na$ requires 383.1471], Figure 4.5. By contrast MS of the 4-*C*-benzyl tetrol showed none of this peak.

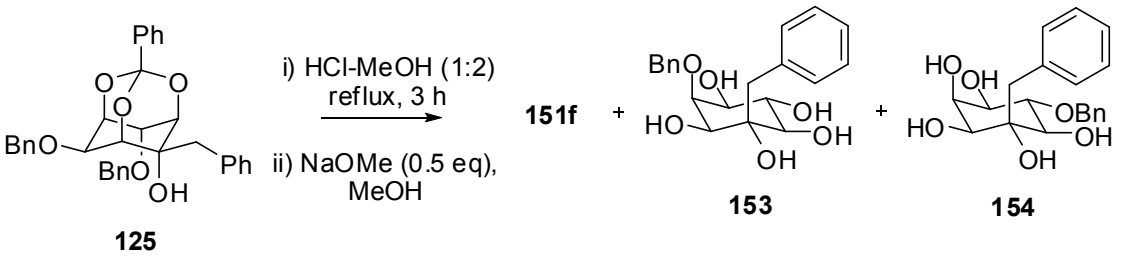


Figure 4.5: Acidic hydrolysis of the 4-*C*-benzyl orthobenzoate generates both the desired tetrol and unwanted pentols

The isolation of the pentol shows that the strongly acidic reaction conditions required to effect hydrolysis were resulting in simultaneous cleavage of the 2- and 6-*O*-benzyl ethers. This explained the loss of mass on purification of the crude tetrols when the much more polar pentol by-product would remain on the silica pad.

The increased resistance to acidic hydrolysis of the 4-*C*-alkyl orthobenzoates compared to their non-alkylated counterparts can be interpreted by the mechanism of 1,3,5-triol orthoester hydrolysis established by Bouab *et al.*,[103] as described in chapter 2, Figure 2.20. In order for the addition of water to occur during hydrolysis, the inositol ring of the intermediate must undergo a chair-boat inversion. This provides sufficient space for the addition of water, and prevents competing recombination to regenerate the starting material. Bouab *et al.*[103] reported that substitutions, such as a phenyl ring, at the bridgehead position result in reduced rates of hydrolysis. Additionally, in the 4-*C*-alkyl orthoester, protonation of any of the 1-, 3- or 5-*O* followed by chair-boat interconversion of **156** to **157** introduces a steric clash of adjacent eclipsing pseudo-axial substituents, of either the 4-*C*-alkyl or 6-*O*-benzyl with the dioxacarbenium moiety. Furthermore, access of water to one face of the carbocation over the inositol ring will be severely hindered by the adjacent axial substituent. Access to the other, less hindered face is also unfavourable, as this will increase the clash with the substituent on the other side to generate **158**. All these factors will result in a shift of the initial equilibria towards the starting material, Figure 4.6.

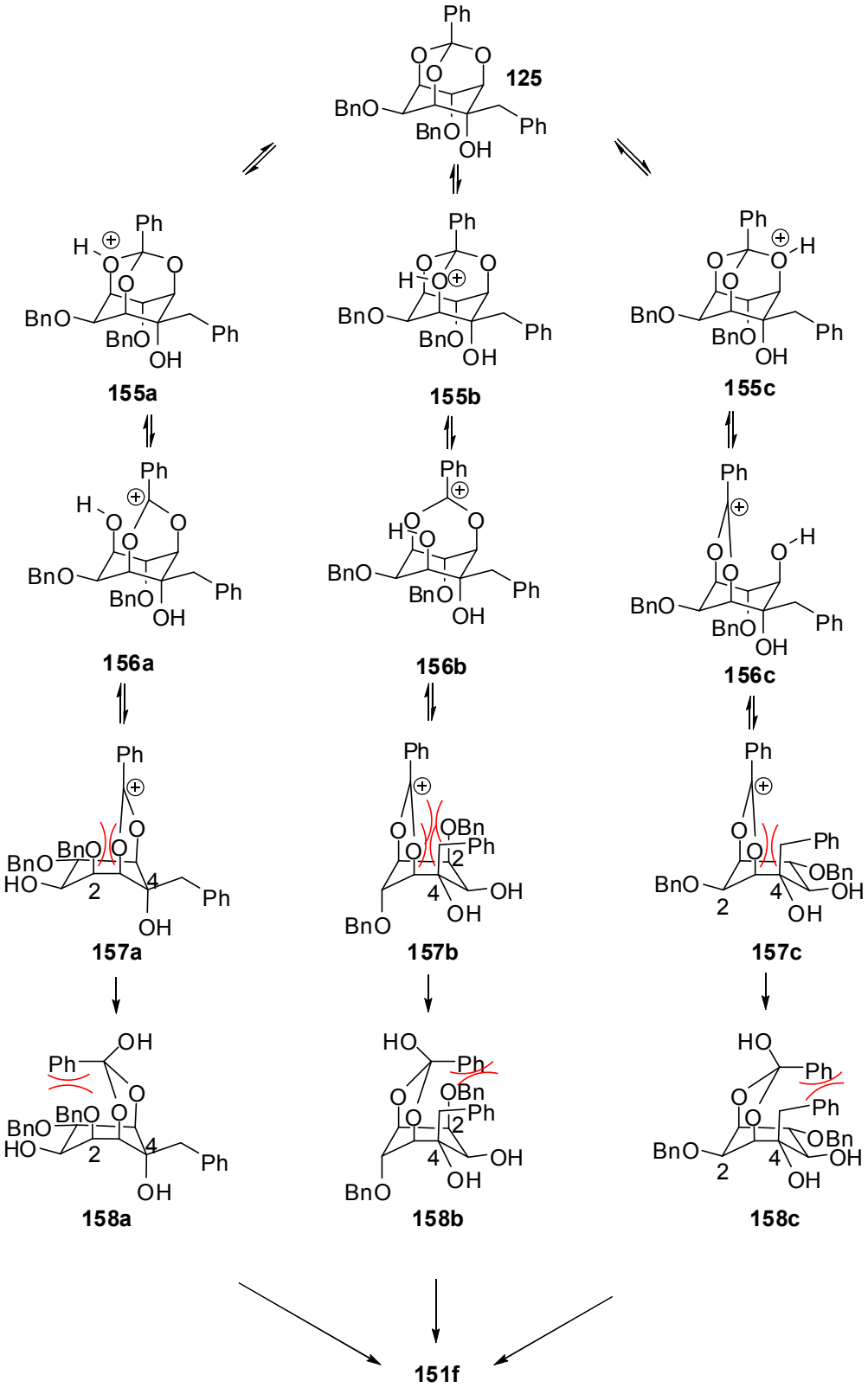


Figure 4.6: Rate determining step of acidic hydrolysis is likely to be hampered in this system due to steric clash

The 4-*C*-alkyl tetrols were fluxional presumably due to the now axial alkyl addition, and this was evident in their ^{1}H-NMR spectra, which are broad, with reduced coupling constants between the 6-*H* and its *anti* 1- and 5-*H* neighbours, indicating a reduced dihedral angle. For 4-*C*-

pentyl tetrol **151c** in the chair conformation a coupling constant of 7-10 Hz would be expected for this ring proton, due to its two antiperiplanar neighbours. However, the measured coupling constant is 3.4 Hz, and an NOE is observed between both the 3- and 5-*H* and the pentyl CH_2 suggesting that non-chair conformations, with the 4-*C*-alkyl group in an equatorial orientation, are also partially occupied.

4.2 Preparation of the orthoformates

The low yields obtained from acidic hydrolysis of the orthobenzoate led us to consider the more widely utilised orthoformate as an alternative 1,3,5-*O* protecting group. It is reportedly more labile to acidic hydrolysis than the corresponding orthobenzoate.[73,100]

Consequently *myo*-inositol 1,3,5-*O*-orthoformate was prepared by transesterification of *myo*-inositol with trimethyl orthoformate. Direct 4-*O*-allylation of the crude material was carried out by addition of 1 eq. sodium hydride followed by allyl bromide,[84] to obtain **31** in an unoptimised yield of 34 %. This avoided the more time consuming reported methods involving preparation of the tribenzoate[68] or chromatography of the crude orthoformate.[70] Benzylation of the remaining 2- and 6-*O* with excess sodium hydride and benzyl bromide generated the fully protected intermediate **32**. Isomerisation of the 4-*O*-allyl proceeded as reported for the orthobenzoate to prepare the 4-*O*-(prop-2-enyl) ether **159**.[84] As predicted by the results of Billington and Gigg,[85] removal of the 4-*O*-(prop-2-enyl) ether under acidic conditions generated both desired product **41** and 2,6-*O*-dibenzyl tetrol resulting from acidic hydrolysis of the orthoformate. However **41** could be regenerated by the reaction of 2,6-*O*-dibenzyl tetrol with trimethyl orthoformate. The alternative use of basic aqueous $KMnO_4$ in methanolic NaOH[86] effected a selective oxidative removal of the prop-2-enyl ether without any loss of the orthoformate.

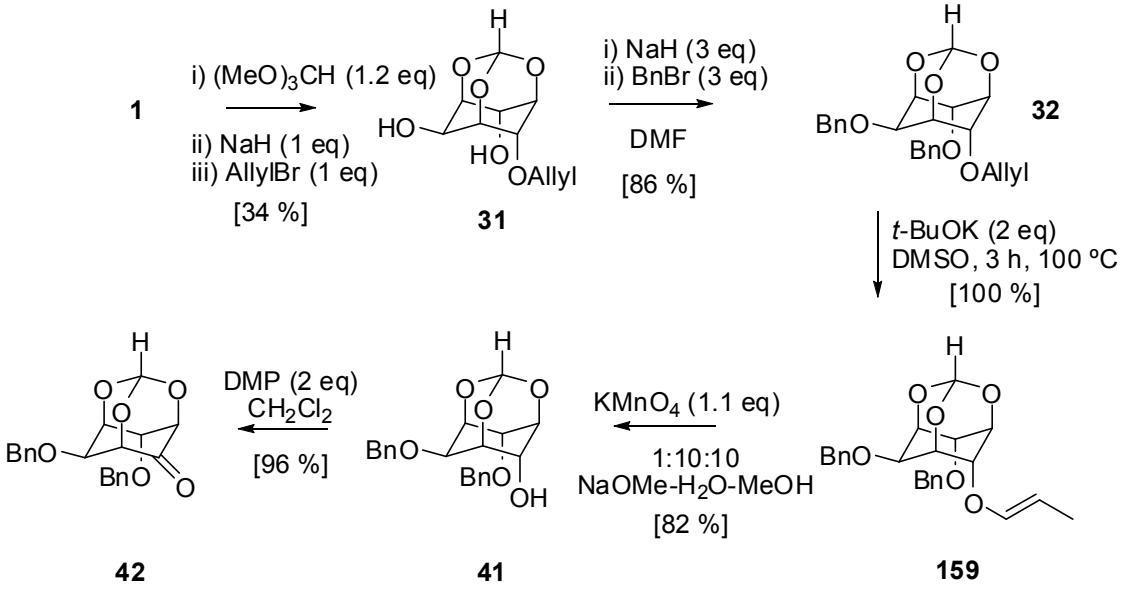


Figure 4.7: Preparation of 2,6,-*O*-dibenzyl inos-4-ose 1,3,5-*O*-orthoformate from *myo*-inositol

Oxidation of the 4-*O*H with Dess Martin periodinane (DMP) generated inos-4-ose **42**, which was converted to the *exo*-methylene oxide using sulfoxonium ylide. *exo*-Methylene oxide **160** underwent the predicted nucleophilic opening with phenyl lithium cuprate to generate 4-*C*-benzyl-2,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthoformate **161**, which was used to test the acidic deprotection of the 4-*C*-alkyl orthoformates, Figure 4.8.

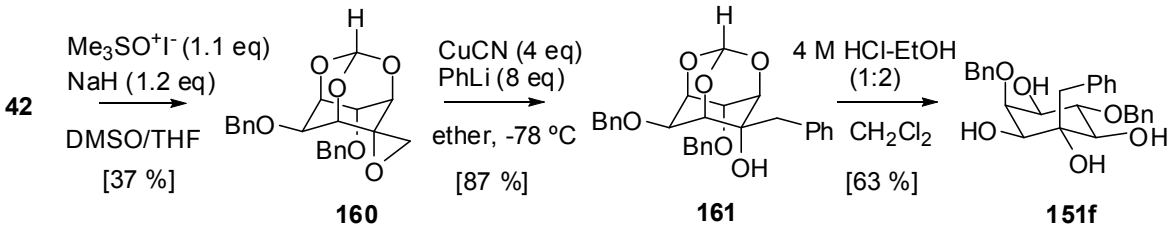


Figure 4.8: Preparation of 4-*C*-benzyl orthoformate from inos-4-ose **42** and subsequent acidic hydrolysis

Similarly to the orthobenzoate, the published conditions for orthoformate hydrolysis [TFA:H_2O (4:1)][100] did not initiate acidic deprotection of 4-*C*-benzyl orthoformate **161**. Upon treatment of **161** with 2 M HCl-EtOH (1:2) some signs of acidic hydrolysis were observed, but after 48 h the conversion was only 50 % complete. Increasing the concentration of HCl to 4 M, increased the rate of reaction. After purification of crude **151f**, the mass recovery was improved (63 % product, 10 % starting material recovered) compared to the 4-*C*-benzyl orthobenzoates and no evidence for 2- or 6-*O*-benzyl ether hydrolysis was observed. Therefore, during future work involving introduction of inositol ring substituents it is advised that the smaller 1,3,5-*O*-orthoformate is used in order to reduce steric retardation of acidic hydrolysis. Unfortunately, unlike the benzoate ester generated by acidic hydrolysis of the orthobenzoate, the presumed formate ester from initial orthoformate hydrolysis is immediately cleaved under the conditions used,[78,100] to generate the tetrol. While this is ideal for the preparation of tetrols for IP_4 head groups, alternative methods would be required to isolate the 1-*O* for preparation of PIP_3 lipid analogues.

4.3 Selective DIBAL-H reduction to prepare natural Ins(1,4,5)P_3

It was hoped that the reduction of the orthobenzoate using excess DIBAL-H could ultimately be used to prepare the 4-*C*-alkyl-2,3,6-*O*-tribenzyl inositols required for the synthesis of 4-*C*-alkyl IP_3 analogues. Reduction of orthoformates and orthobenzoates with limited DIBAL-H to generate the corresponding 1,3-*O*-acetals by hydride insertion into the 5-*O*-C bond is known.[105] It was hoped that a second hydride delivery to the 1-*O*-C bond would generate the 3-*O*-benzyl ether. Such a reduction was not previously reported and therefore initial test reactions were carried out on 2,4,6-*O*-tribenzyl orthobenzoate **162**.

Symmetrical 2,4,6-*O*-tribenzyl orthobenzoate **162** was prepared by exhaustive benzylation of *myo*-inositol 1,3,5-*O*-orthobenzoate. Use of limited DIBAL-H failed to replicate the yields of Maurali *et al.*[105] who report isolation of the bridging 1,3-*O*-benzylidene acetal in 97% yield from 2,4,6-*O*-tribenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **162**. Despite multiple attempts, addition of limited DIBAL-H produced a mixture of the fully reduced **164**, the desired bridging benzylidene acetal **163** and some remaining starting material, Figure 4.9.

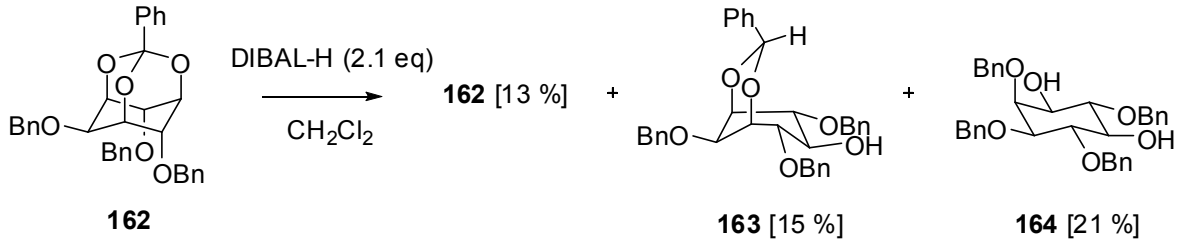


Figure 4.9: Partial reduction of the tribenzyl generated a mixture of products

Only the symmetrical benzylidene acetal was observed, which is consistent with the literature precedent that hydride delivery occurs initially to the 5-*O*-C bond,[106] and the subsequent generation of only the asymmetric 1,2,4,6-*O*-tetrabenzyl product.

Upon treatment of **162** with excess DIBAL-H, total reduction of the orthobenzoate to 1,2,4,6-*O*-tetrabenzyl inositol **164** was observed. For the 2,4,6-*O* tribenzyl orthobenzoate, the two products formed from a second hydride insertion into the 1-*O* or 3-*O* bond of the benzylidene acetal are identical by symmetry, occurring by definition in a 1:1 ratio. Since the 5-*O*-benzyl product would be symmetrical, and therefore easily identified, the regiochemistry of reduction to the 1(3)-*O*-benzyl was confirmed by the generation of asymmetrical **164**, Figure 4.10.

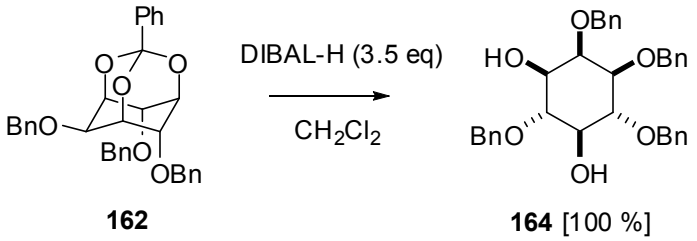
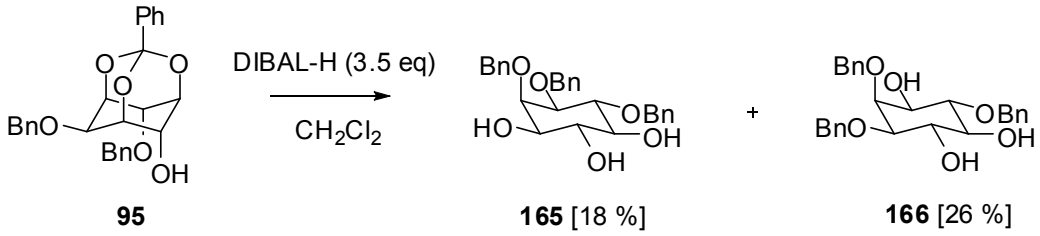


Figure 4.10: Total reduction of 2,4,6-*O*-tribenzyl orthobenzoate generates one product

In order to generate the desired IP_3 precursors, it was hoped that the second hydride insertion could be directed to the 1-*O* of an orthoester with asymmetric protection. This would preferentially generate the desired 3-*O*-benzyl ether. Such regiocontrol would also be of great benefit in the preparation of natural Ins(1,4,5)P_3. It was postulated that the 4- and 6-*O* protecting groups could be used to direct the second DIBAL-H delivery.

For an asymmetrical material, such as 2,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **95**, the second hydride insertion at either the 1- or 3-*O* is expected to generate two distinct products assuming initial attack at the 5-*O*; 1,2,6-*O*-tribenzyl-*myo*-inositol **165** and desired 2,3,6-*O*-tribenzyl-*myo*-inositol **166**, Figure 4.11.



95 **165** [18 %] **166** [26 %]

Figure 4.11: Treatment of **95** with excess DIBAL-H generates two products

It was postulated that on treatment of **95** with excess DIBAL-H, initial coordination of the first equivalent of DIBAL-H to the 4-*O*H may present a larger steric hindrance to hydride delivery at the adjacent 3-*O* than hindrance of the 1-*O* by the 6-*O*Bn, thereby directing the reduction of the intermediate 1,3-*O*- benzylidene acetal to give a 3-*O* benzyl ether. This would provide an expedient method for directing the regioselectivity of reduction, as the bulky 4-*O*-DIBAL-adduct is removed in the work up. However the ratio of products obtained showed that there was little selectivity for the desired 3-*O*Bn (Entry A, Table 4.2). It was not possible to further influence the ratio of products despite variations in the temperature and rate of addition of DIBAL-H.

Table 4.2: Ratio of crude products in ^{1}H-NMR of 1- and 3-*O*-benzyl ethers from DIBAL-H reduction

Entry	Starting Material	4-*O*-R	Ratio of products		
			3-*O*-Bn		1-*O*-Bn
A	**95**	H	4	:	3
B	**162**	Bn	1	:	1
C	**167**	Tbdms	4	:	5
D	**168**	Tbdps	4	:	5

Noting that there was a slight preference for desired **166** we hoped to further differentiate between the two regioisomers by introducing bulkier temporary protecting groups at the 4-*O*. Unfortunately, upon treatment of 4-*O*-Tbdms (**167**) and 4-*O*-Tbdps (**168**) with excess DIBAL-H both reactions generated two diols, **169** and **170**, and **171** and **172** respectively, the result of both 1- and 3-*O*-C hydride insertion, Figure 4.12.

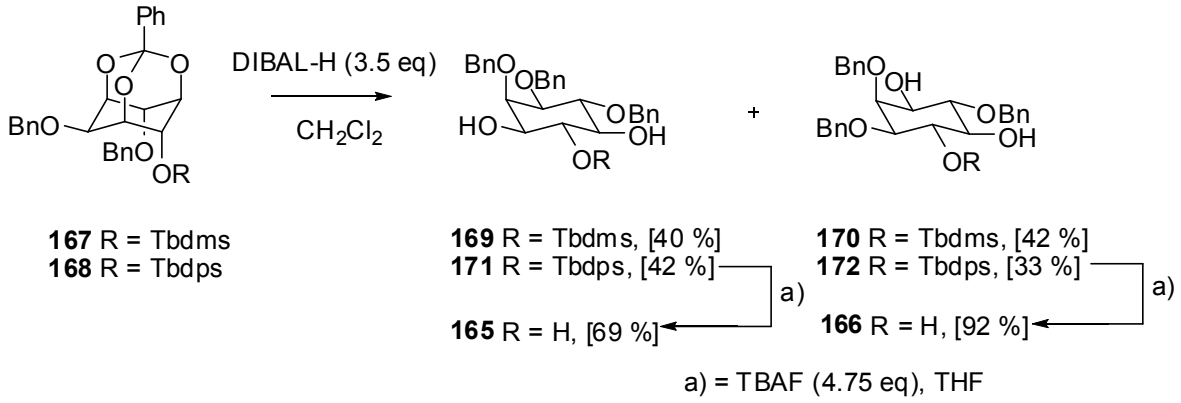


Figure 4.12: Treatment of **167** and **168** with excess DIBAL-H

^{1}H-NMR of the crude material showed a slight preference for the 1-*O* benzyl products (Table 4.2, C and D). The disappointing lack of selectivity may be due to the repulsion of the bulky 4-*O*-silyl group by the adjacent 5-*O*-DIBAL adduct in the intermediate benzylidene acetal; such bulky groups have been shown to favour the 1,2-diaxial conformation, forcing the intermediate back into a chair-like shape,[146] Figure 4.13.

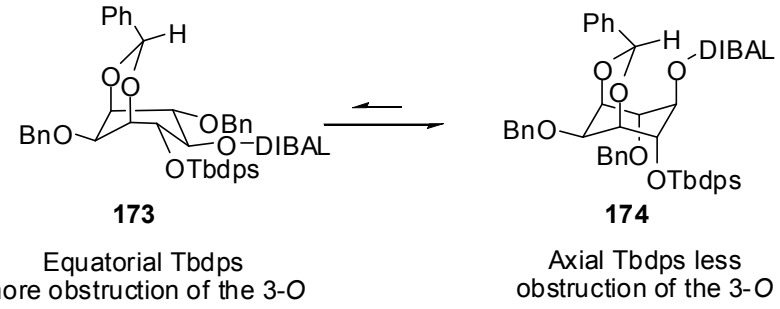


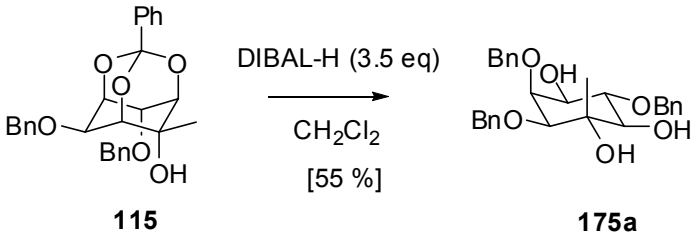
Figure 4.13: Bulky 4-*O*-silyl intermediates **173** may be forced back into chair configuration **174** due to 5-*O*-DIBAL adduct

Despite the lack of selectivity observed, an advantage of preparing the 4-*O*-Tbdms and -Tbdps derivatives was that the diols were considerably easier to separate using flash chromatography than the corresponding triols from reduction of 2,6-*O*-dibenzyl orthoester **95**. This was reflected in the isolated yields of the two regioisomers in each case (40 and 42 % recovery for 4-*O*-Tbdms diols *cf.* 26 and 13 % for triols). The identities of **171** and **172** were confirmed by removal of the 4-*O*-Tbdps using TBAF to afford triols **165** and **166**, for which the regiochemistry had been unambiguously defined by COSY.

4.4 Reduction of 4-*C*-methyl orthobenzoate with DIBAL-H is regioselective

It was demonstrated in section 4.3 that addition of excess DIBAL-H to the 1,3,5-*O*-orthobenzoate generates a benzyl ether. However attempts to direct the regioselectivity of the second hydride delivery to the 1,3-*O*-benzylidene acetal were unsuccessful. It was hoped that the complete reduction of the 4-*C*-methyl orthobenzoate **115** would show increased regioselectivity due to the equatorial 4-*C*-methyl group.

Upon addition of excess DIBAL-H to **115** a single product was generated, **175a**. Of the three possible regioisomers of the benzyl ether the desired 3-*O* regioselectivity was confirmed by the clear NOESY between the 2-, 3- and 6-*H* and their benzyl-CH_2 signals in the ^{1}H-NMR, Figure 4.14.



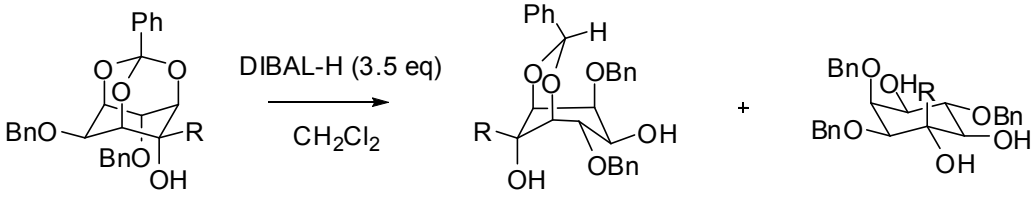
115 **175a**

Figure 4.14: Addition of DIBAL-H to 4-*C*-methyl derivative **115** generates one product

During the initial hydride reduction at the 5-*O* on inositol orthoesters lacking a 4-*C*-alkyl group, it is the steric effect of the equatorial 2-*O* benzyl ether which is assumed to direct the bulky DIBAL-H away from the 1- and 3-*O*.[105] The 4-*C*-methyl derivative **115** has both an equatorial 2-*O*-benzyl and the equatorial 4-*C*-methyl, which should increase the steric hindrance at the 3-*O* substantially. It would be informative to isolate the intermediate benzylidene acetal as the position of initial hydride delivery would indicate which of the two equatorial groups had the greater steric influence. However, despite multiple attempts, it was not possible to isolate any benzylidene acetal from the addition of limited DIBAL-H to **115**. Therefore it cannot be determined in which order the 1- and 5-*O*-C bonds undergo hyride insertion.

4.5 Treatment of larger 4-*C*-alkyl orthobenzoates with DIBAL-H

Following the totally selective reduction of 4-*C*-methyl orthobenzoate **115** to the desired 2,3,6-*O*-tribenzyl ether, it was anticipated that larger 4-*C*-alkyl substituents would produce a similar directing effect. Addition of excess DIBAL-H to **136**, **133**, **125**, **145** and **144** produced two products; a single benzylidene acetal isomer and the 2,3,6-*O*-tribenzyl triol, Figure 4.15 and Table 4.3.



136 R = ethyl
133 R = pentyl
144 R = neopentyl
125 R = benzyl
145 R = (2-naphthyl)methyl

176b R = ethyl
176c R = pentyl
176e R = neopentyl
176f R = benzyl
176g R = (2-naphthyl)methyl

175b R = ethyl
175c R = pentyl
176e R = neopentyl
175f R = benzyl
175g R = (2-naphthyl)methyl

Figure 4.15: Addition of DIBAL-H to the larger 4-*C*-alkyl derivatives generates two products

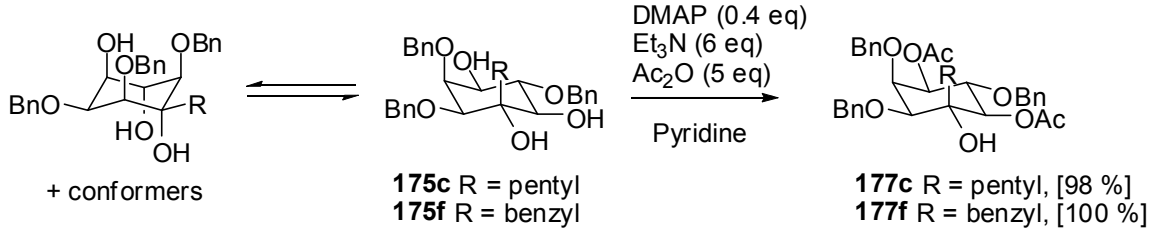
With the exception of 4-*C*-ethyl orthobenzoate **136** (Table 4.3, entry 2), longer reaction times and addition of further DIBAL-H did not force the reaction to completion and both the acetal and triol were isolated.

Table 4.3: Isolated yields from addition of DIBAL-H to 4-*C*-alkyl orthobenzoates

Entry	Starting Material	4-*C* Alkyl Derivative	Yield of Benzylidene Acetal, 176 (%)	Yield of 2,3,6-*O*-Triol, 175 (%)
1	**115**	methyl	0	55
2	**136**	ethyl	0(19)*	61(32)*
3	**133**	pentyl	13	54
4	**144**	neopentyl	58	12
5	**125**	benzyl	28	39
6	**145**	(2-naphthyl)methyl	42	29

*Ethyl can be either forced to completion by longer reaction times (48 h), or if both compounds are desired, can be stopped earlier (12 h) yields shown in parentheses.

The fluxional nature of these compounds did not permit clear assignment of regiochemistry using ^{1}H-NMR. Therefore the 1,5-*O*-diacetyl derivatives **177c** and **f** were prepared from 4-*C*-pentyl and 4-*C*-benzyl triols **175c** and **175f**. No acetylation was observed on the more hindered tertiary 4-*O*H, Figure 4.16.



+ conformers

175c R = pentyl
175f R = benzyl

177c R = pentyl, [98 %]
177f R = benzyl, [100 %]

Figure 4.16: Acetylation of the 1- and 5-*O* confirms triol regiochemistry

Locking the compounds into a single chair conformation, the ^{1}H-NMR of diacetates **177c** and **f** was sharp, with easily visible splitting patterns and measurable coupling constants. The 4-*C*-benzyl 1- and 5-*H* resonated at 5.01 (1H, d, *J* 9.8, Ins 5-*H*) and 4.95 (1H, bd, *J* 7.8, Ins 1-*H*) ppm respectively, roughly 1 ppm higher than the unacylated Ins-*H* shifts of the remaining 2-, 3-, and 6-*H*. The 3-*H* [for 4-*C*-benzyl, δ 3.64 (1H, d, *J* 2.4)] was not acetylated in either case, indicating that this was the location of the benzyl ether. This confirmed the regiochemistry of reduction to the desired 2,3,6-*O*-tribenzyl triol.

The benzylidene acetals **176b**, **c**, **e-f** isolated were stable to further DIBAL-H reduction. The two products were easily separated using flash chromatography and the less polar acetal was found to be a single regioisomer. Of the three possible benzylidene acetals which could be generated from initial hydride delivery only 3,5-*O*-benzylidene acetals **176** were generated, Figure 4.17.

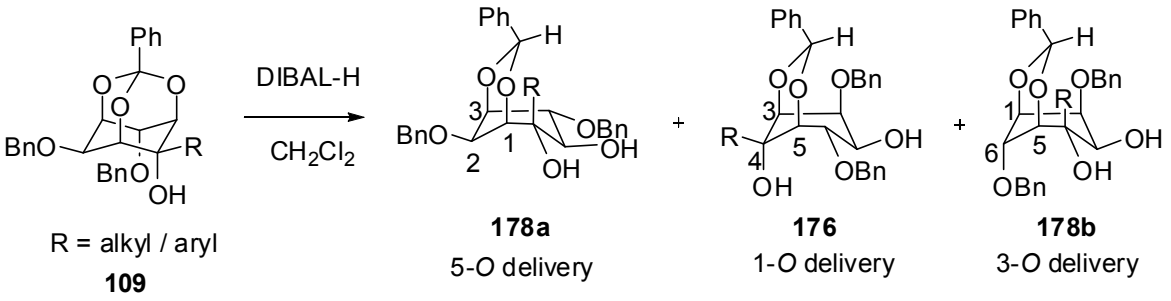


Figure 4.17: Hydride delivery to the orthobenzoate can occur at one of three positions

The ^{1}H-NMR for these species have a clear quartet in the inositol region, which exchanges to a triplet on addition of D_2O [e.g. for 4-*C*-ethyl benzylidene acetal δ 4.46 (1H, q, *J* = 7.7 Hz, ex → t)]. Such a resonance could only be generated by **176** where the ring proton with a free hydroxyl has two neighbouring protons, to generate a triplet, Figure 4.18.

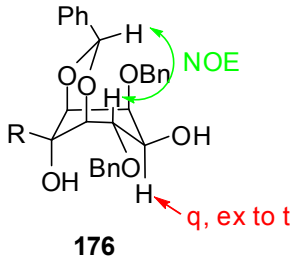


Figure 4.18: ^{1}H-NMR splitting and NOE confirm identity of benzylidene acetal

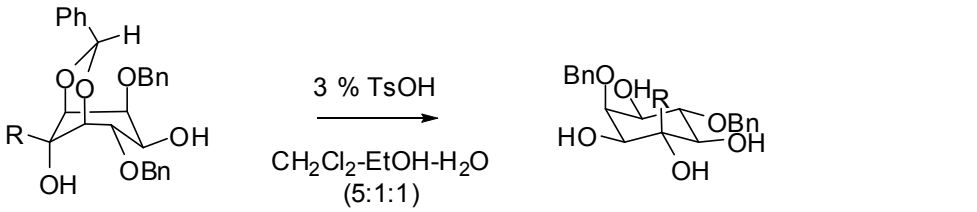
This was corroborated by NOE of the benzylidene acetal bridgehead proton to the 6-*H* ring proton Figure 4.18. Such an interaction would not be possible in **178b**. This unexpected result demonstrates that with the additional 4-*C*-alkyl group, the mechanism for DIBAL-H reduction may now proceed by initial hydride insertion to the 1-*O*-C bond, rather than the 5-*O*-C bond. However,

the stability of **176** to a large excess of DIBAL-H and long (24 h) reaction time, as the 4-*C*-alkyl substituent size increases, indicates that the 3-and 5-*O* of the benzylidene acetals are very inaccessible. Indeed, treatment of 4-*C*-benzyl benzylidene acetal **176f** with excess DIBAL-H at 50 ºC for 24 h only showed traces of conversion to the desired triol by ^{1}H-NMR, suggesting that this acetal is quite stable towards further reduction. In each case there was DIBAL-H remaining when the reaction was quenched, as violent reaction was observed upon dropwise addition of H_2O. It is therefore unlikely that the isolated triol derives from **176**. Instead it is postulated that **178a** from 5-*O*-C reduction is formed transiently, as opposed to **178b** which is unlikely to form as the DIBAL-H would be required to coordinate between the two equatorial substituents. Unobserved benzylidene **178a** is then reduced rapidly by a second hydride insertion at the 1-*O*-C bond. As the size of the 4-*C*-alkyl substituent increased the relative proportion of benzylidene acetal compared to the triol also increased, Table 4.3. This suggests that for smaller 4-*C*-alkyl groups both **178a** and **176** are easily reduced by DIBAL-H, but for larger 4-*C*-alkyl substituents access to the 5-*O* is much more restricted leading to accumulation of **176**. This regiocontrol suggests that larger 4-*C* alkyl groups are more sterically demanding than the 2-*O* benzyl ether.

The ^{1}H-NMR of the 3,5-*O*-benzylidene acetals is characteristic. No coupling is observed between the adjacent Ins 5-*H* and Ins 6-*H* indicating that they have a dihedral angle of *ca.* 90 degrees to each other,[129] rather than the 120 º angle suggested by their axial-equatorial relationship in a strictly boat conformation. Also, in a strictly boat form, the 1-*H* would couple with a large coupling constant (8-10 Hz) to the anti-periplanar 6-*H* and with a smaller coupling constant (1-4 Hz) to the gauche 2-*H*, forming a double doublet. However the **176f** 1-*H* resonates as an apparent triplet [δ 4.46 (1H, t, J = 7.7 Hz]. These unusual dihedral angles suggest that the acetals are twisted out of the boat conformation – presumably to relieve steric clashes such as the pseudo-axial 2-*O* benzyl adjacent to the 3,5-*O*-benzylidene acetal and the 1,4-diaxial interaction of the 1-*H* and 4-*O*H.

4.6 Removal of 3,5-*O*-benzylidene acetals: An alternative route to 4-*C*-alkyl tetrols

Initial attempts to record ^{1}H-NMR spectra of the benzylidene acetals using $CDCl_3$ showed signs of decomposition after 2 h. Comparison to the relevant 2,6-*O*-tetrols confirmed that this was a result of acidolysis of the 3,5-*O*-benzylidene acetal, demonstrating the sensitivity of this group to acidic conditions. The benzylidene acetal generated from the orthobenzoate is much more labile than the corresponding formate acetal generated from the orthoformate.[147] Treatment of 4-*C*-alkyl benzylidene acetals **176e**, **f** and **g** with 3 mol% *p*-TsOH in H_2O-EtOH-CH_2Cl_2 cleanly afforded 4-*C*-alkyl tetrols **151e**, **f** and **g**, which were separated from the resulting benzaldehyde by-product by flushing though a pad of flash silica, Figure 4.19.



176e R = neopentyl
176f R = benzyl
176g R = (2-naphthyl)methyl

151e R = neopentyl, [56 %]
151f R = benzyl [75 %]
151g R = (2-naphthyl)methyl, [43 %]

Figure 4.19: Removal of the 3,5-*O*-benzylidene acetal to generate tetrols

Reduction of the orthobenzoate with DIBAL-H to selectively generate both the required triols and tetrols for larger *C*-alkyl derivatives is particularly useful in simultaneously preparing 4-*C*-alkyl IP_3 and IP_4 libraries from a common precursor. Due to the unexpected difficulties of using acidic hydrolysis to effect removal of the orthobenzoate (see section 4.1) this route was adopted as the major pathway for generating 4-*C*-alkyl tetrols for use in further steps.

4.7 Concluding Remarks

Acidic deprotection of the orthobenzoate required considerably harsher conditions than the corresponding unalkylated orthoesters, due to the combination of the orthobenzoate head group and 4-*C*-alkyl substituent causing reduction of the rates, presumably for steric reasons. The harsh conditions required to initiate hydrolysis of the orthobenzoate also caused partial hydrolysis of the benzyl ethers. The corresponding 4-*C*-benzyl orthoformate was prepared, and was more easily removed without evidence of 2 and 6-*O*-benzyl ether deprotection.

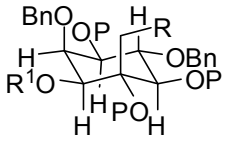
Using steric bulk on the 4-*O* in an attempt to influence the direction of DIBAL-H reduction for preparation of natural IP_3 was unsuccessful. However, the reduction of 4-*C*-alkyl orthobenzoates with DIBAL-H proceeded regioselectively generating the desired 4-*C*-alkyl 2,3,6-*O*-tribenzyl triols. DIBAL-H reduction of 4-*C*-ethyl and larger substituents led to the concurrent isolation of the benzylidene acetal. It was demonstrated that this was labile under mildly acidic conditions to easily afford the desired 4-*C*-alkyl 2,6-*O*-tetrols for IP_4 preparation by an alternative route. The discovery that both IP_3 and IP_4 precursors can be simultaneously generated using DIBAL-H reduction led to this being the primary method for obtaining these compounds for further elaboration.

Chapter 5: Phosphorylation and deprotection of 4-*C*-alkyl triols and tetrols

The selectively protected 4-*C*-alkyl *myo*-inositol triols and tetrols, the preparation of which is described in the previous chapter were now ready for polyphosphorylation. Unlike in the natural systems or published syntheses, the key challenge was to effect phosphorylation of a polyol which included a tertiary centre. As discussed previously (section 2.5), the principle challenge during polyphosphorylations of vicinal diols is avoiding cyclisation of the intermediate phosphites with the consequent loss of yield. The reagent of choice would be furnished with benzyl groups to permit global deprotection of both the phosphate esters and the 2-, 3- and 6-*O*-benzyl ethers on the ring hydroxyls in one step.

In the final steps of phosphorylation and deprotection, the ^{31}P-NMR becomes an invaluable analytical tool. Each phosphate introduced gives a single signal in the proton decoupled ^{31}P-NMR. Phosphorylation of these derivatives introduces a new dimension into the ^{1}H-NMR; as ^{31}P is also a spin ½ nucleus, ^{1}H-^{31}P coupling is observed. This is useful to identify the regiochemistry of phosphorylation although, due to the size of the 3-bond P-H coupling (6-9 Hz) which is roughly the same as the anti-periplanar coupling constant, care must be taken not to confuse the two.

Table 5.1: Additional splitting of protons in ^{1}H-NMR for 4-*C*-alkyl phosphorylated derivatives due to ^{31}P coupling.



R = alkyl
P = phosphate
112 R^1 = H
113 R^1 = P

Proton	Orientation	Splitting	^{31}P-coupling	Observed peak
1-H	Axial	dd	Yes	td
2-H	Equatorial	t	No	t
3-H	Axial	d	IP_3 No	d
			IP_4 Yes	dd
4-H	-	-	-	-
5-H	Axial	d	Yes	t
6-H	Axial	t	No	t

The presence of multiple benzyl peaks in the fully protected species, each with doublets in the 5 ppm region, would be expected to lead to complex spectra for these intermediates, especially

if they are fluxional. However this intermediate can be characterised using ^{31}P-NMR and the removal of side products or phosphorylating reagent should be clear from this spectrum.

Once the benzyl groups are removed to generate the free 4-*C*-alkyl inositol tri- or tetraphosphate, there should be no peaks above 5 ppm in the ^{1}H-NMR as there will be no remaining aromatic groups (except, of course, if they are contained in the 4-*C*-alkyl substituent), and the inositol region of the ^{1}H-NMR spectra will be free of benzylic CH_2 peaks. The distinctive coupling of each remaining inositol proton should then be visible.

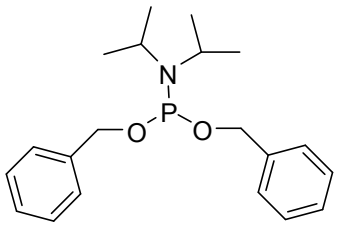
The ^{13}C-NMR is also affected by the introduction of ^{31}P nuclei. ^{31}P coupling affects carbon centres two and three bonds away, splitting them into doublets with coupling constants of 3-6 Hz, with no clear distinction between 2- and 3-bond coupling, Table 5.2.

Table 5.2: Observed ^{13}C-NMR resonances after phosphorylation and deprotection

Carbon	Peak Type	IP_3 splitting	IP_4 splitting
1-C	C-H	d	d
2-C	C-H	d	t/dd
3-C	C-H	d	t/dd
4-C	Quaternary	t/dd	~q
5-C	C-H	t/dd	t/dd
6-C	C-H	t/dd	t/dd

5.1 Phosphorylation of a tertiary centre

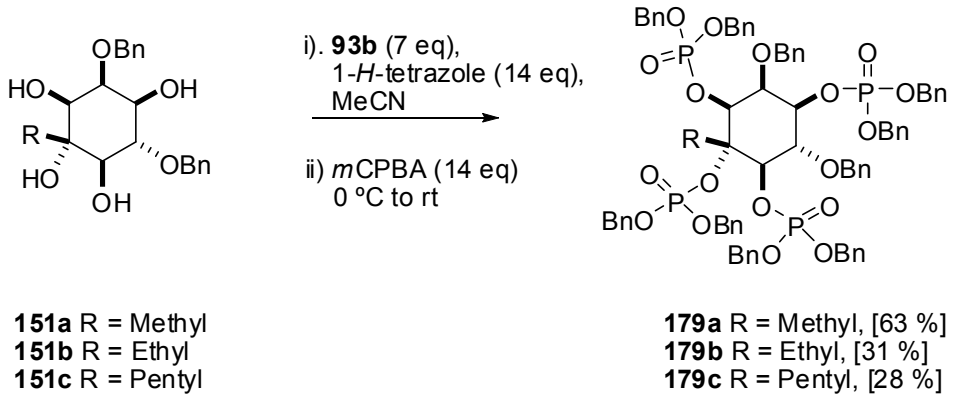
As a starting point to test the phosphorylation of the newly introduced tertiary centre *N,N*-diisopropyldibenzyl phosphoramidite, **93b**, was employed, Figure 5.1. This commercially available reagent is reported by Yu and Reid[110] to permit polyphosphitylation under mild conditions. Furthermore the use of a dibenzyl phosphitylating reagent has the advantage of global deprotection by catalytic hydrogenation to obtain the final product.



93b

Figure 5.1: *N,N*-diisopropyldibenzylphosphoramidite, **93b**

Phosphitylation of 4-*C*-methyl, -ethyl and -pentyl tetrols in MeCN with 1-*H*-tetrazole as activator, followed by oxidation with *m*CPBA furnished the three 1,3,4,5-*O*-tetrakis (dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-alkyl-*myo*-inositols **179a-c**, Figure 5.2.



151a R = Methyl
151b R = Ethyl
151c R = Pentyl

179a R = Methyl, [63 %]
179b R = Ethyl, [31 %]
179c R = Pentyl, [28 %]

Figure 5.2: Phosphorylation of 4-*C*-methyl, ethyl and pentyl tetrols

Tetrazole is used as an activator in phosphitylations with phosphoramidies. The mechanism is shown in Figure 5.3.

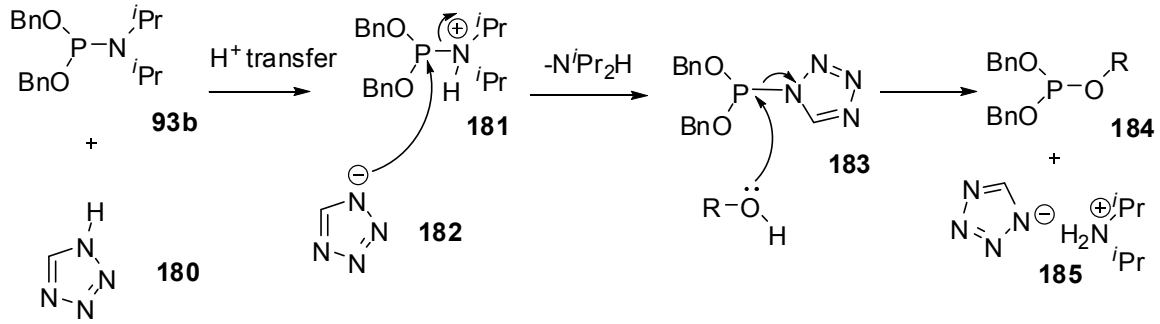


Figure 5.3: Mechanism of phosphitylation using *N,N*-diisopropyldibenzyl phosphoramidite and 1-*H*-tetrazole

H-Phosphonate, formed by hydrolysis of the phosphoramidite **93b** [δ_P 7.06 ppm, δ_H 6.99 (d, *J* 710 Hz)] on addition of the hydrous oxidising agent, was difficult to separate from the product using column chromatography. If the P-H bond of the H-phosphonate were oxidised *in situ* to the phospho-diester salt, this by-product would be easy to remove. However, mildly acidic *m*CPBA does not tautomerise the oxidatively stable P(V) of H-phosphonates to the P(III) phosphite di-ester isomer and so cannot oxidise it. Instead, it was easily removed by oxidation of the crude reaction mixture under basic conditions where H-phosphonate **187** tautomerises to the phosphite diester **188**, Figure 5.4.

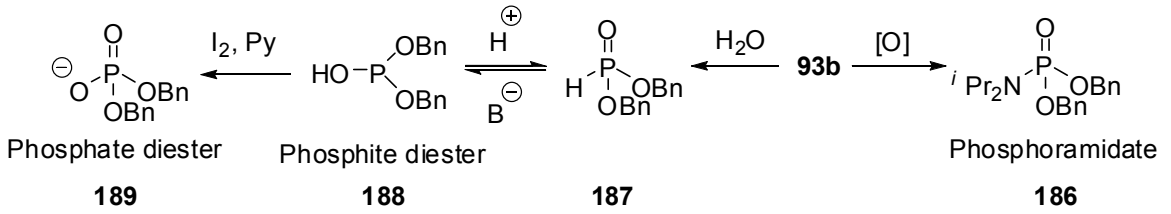


Figure 5.4: By-products in the phosphorylation due to oxidation and hydrolysis of the phosphoramidite, **93b.**

Treatment with 1 M I_2 in H_2O-THF-Py (1:7:2 v/v), followed by extraction of the crude material into $CHCl_3$, leaving the phosphate diester in the aqueous phase. Following oxidation, the remaining debris from the phosphorylating reagent was easily separated using flash chromatography, Figure 5.5.

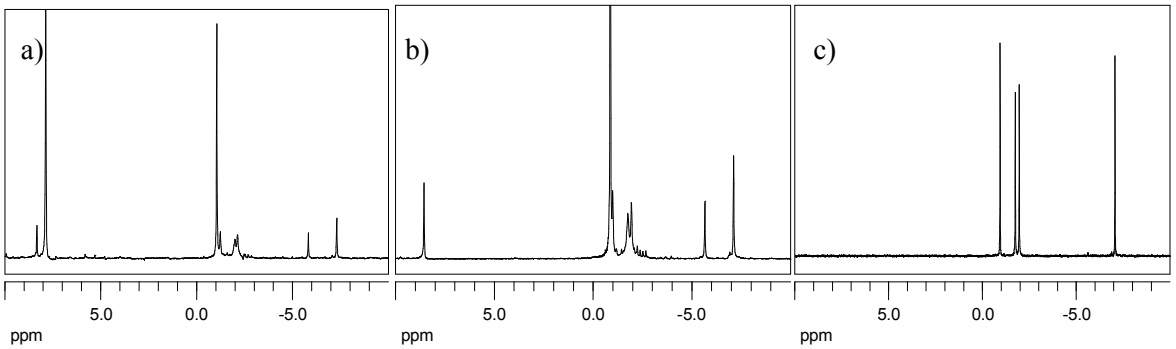


Figure 5.5: Purification 4-*C*-ethyl IP_4 after phosphorylation; a) ^{31}P-NMR of crude material; b) Removal of H-phosphonate by I_2 oxidation; c) Pure compound after column chromatography.

In each case ^{31}P-NMR clearly showed the formation of 4 peaks (for example, 4-*C*-methyl, δ_P -1.46, -1.65, -1.86 and -6.30 ppm). For each fully protected 4-*C*-alkyl IP_4, the tertiary centre had a characteristic shift in the ^{31}P-NMR around -7 ppm upfield from the three secondary centres. The clean conversion from these reactions is clearly demonstrated by comparison of the crude ^{31}P-NMR from reaction mixtures with and without the tetrol, Figure 5.6. The additional peaks present in the crude material are just those from the excess phosphitylating reagent.

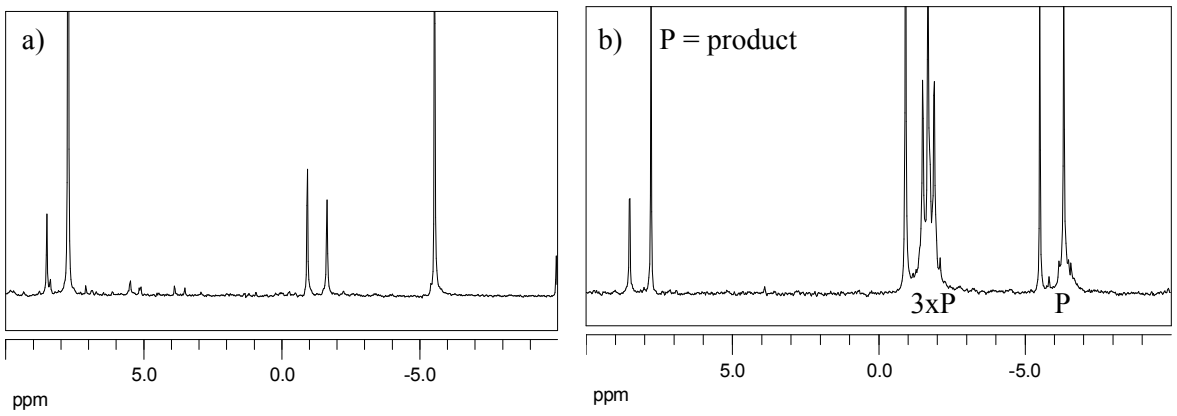


Figure 5.6: a) ^{31}P-NMR of the reagent mixture without tetrol **151a**; b) ^{31}P-NMR of crude material from phosphorylation of 4-*C*-methyl tetrol **151a**

Phosphitylation of bulkier 4-*C*-benzyl and -neopentyl tetrols using **93b**, followed by oxidation with *m*CPBA, generated a mixture of species, identified by ^{31}P-NMR of the crude material, Figure 5.7.

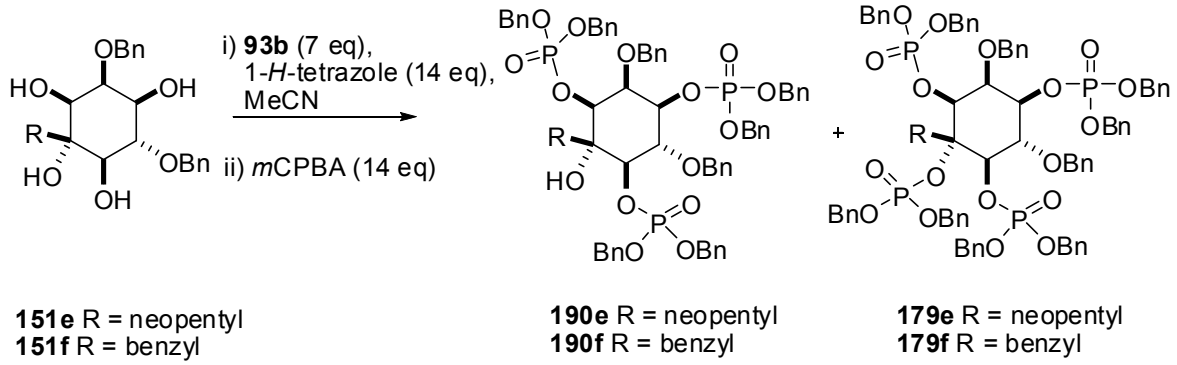
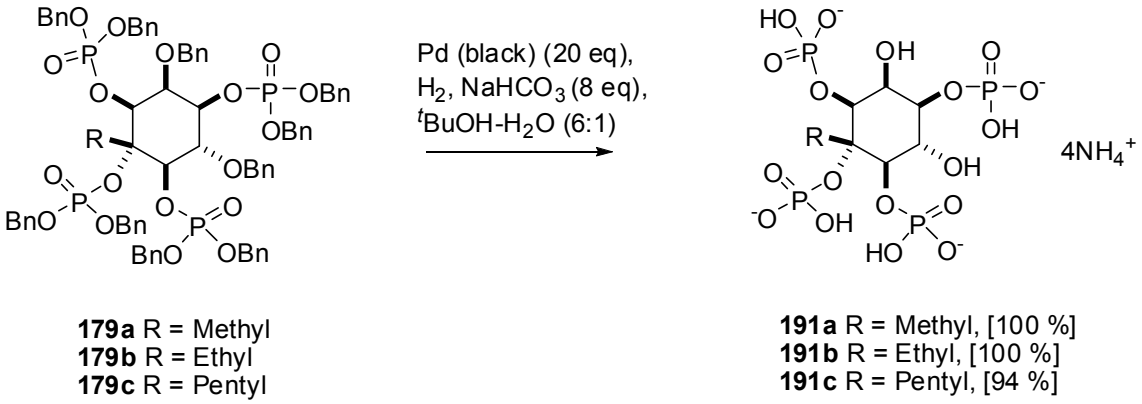


Figure 5.7: Phosphorylation of 4-*C*-benzyl and -neopentyl tetrols is incomplete

The highly similar tri- and tetraphosphorylated species were not separable by flash chromatography, and were only visible as one spot by TLC. However, isolation of some triphosphate from the 4-*C*-benzyl reaction mixture confirmed that the two observed species were 4-*C*-benzyl-1,3,4,5-tetraphosphate and -1,3,5-triphosphate resulting from incomplete phosphorylation at the tertiary 4-*O*H. The regiochemistry of phosphorylation of the triphosphate was easily ascertained by the absence of a ^{31}P peak in the -6 to -7 ppm region [MS (ES^+) *m/z* (%) found triphosphate $[M+H]^+$ 1231 (53), $[M+Na]^+$ 1253 (36), δ_P -0.34, -1.24, -1.76 ppm and tetraphosphate $[M+H]^+$ 1491 (100), $[M+Na]^+$ 1513 (56), δ_P -1.53, -3.05 (2P), -7.09 ppm]. However, it was encouraging to see that limited phosphorylation of the 4-*O*H had occurred giving hope that a more reactive reagent would overcome this problem. Precipitation during the reaction, presumed to be diisopropyl ammonium tetrazolide meant that it was not possible to increase the concentration of the reaction while maintaining stirring, to attempt to push the phosphorylation to completion.

1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-4-*C*-alkyl-*myo*-inositols **179a-c**, were globally deprotected with Pd(black) under an atmosphere of H_2, in BuOOH-H_2O to prepare the three 4-*C*-alkyl IP_4 analogues **191a-c**, Figure 5.8.



179a R = Methyl
179b R = Ethyl
179c R = Pentyl

191a R = Methyl, [100 %]
191b R = Ethyl, [100 %]
191c R = Pentyl, [94 %]

Figure 5.8: Global deprotection of ten benzyl groups to generate 4-*C*-methyl, -ethyl and -pentyl IP_4

The reactions were buffered by the addition of 8 eq. of $NaHCO_3$ in order to neutralise the free acids generated during deprotection, preventing any reduction in the pH which might lead to cyclisation of the phosphates and subsequent loss of material. A concentrated aqueous solution of the crude material was passed through a short column of DOWEX 50WX8-200 H^+ resin to remove complexed Pd^{2+}. Acidic fractions were combined and neutralised with aqueous ammonia before lyopholising. In each case, the ^{31}P-NMR had 4 distinct peaks and a well resolved ^{1}H-NMR permitted individual peaks to be identified and assigned by both splitting pattern and COSY cross-peaks, Table 5.3.

Table 5.3: Assignment of 4-*C*-methyl, -ethyl and -pentyl IP_4 final compounds compared to natural IP_4

Entry	4-*C*-alkyl substituent	δ_P (ppm)	6-*H*, t	
			δ_H (ppm)	*J* (Hz)
1	Methyl	1.74, 1.38, 0.83, -2.35	3.6	9.9
2	Ethyl	1.87, 0.71, 0.27, -4.03	3.77	8.8
3	Pentyl	1.88, 0.65, 0.18, -4.10	3.83	9.0
4[107]	H	1.49, 1.13, 0.59, 0.42	3.77	9.6

In each case, ^{1}H-NMR confirmed that the 4-*H* had been substituted; the 3-*H* all resonate as double doublets, rather than the natural double triplet, and the 5-*H* as triplets, rather than the natural triple doublet or quartet. The stereochemistry of the 4-*C*-alkyl group in the final product was confirmed by observation of an NOESY interaction between the 6-*H* and the 4-*C*-CH_2 for both 4-*C*-methyl and -pentyl IP_4. It is also encouraging to note that these 4-*C* substituted derivatives occupy a naturalistic chair conformation. This is evident from the coupling constants in which the

6-*H* couples to the pair of adjacent protons with near typical anti-periplanar values (Table 5.3, 6-*H* *J* values all between 8-10 Hz), Figure 5.9.

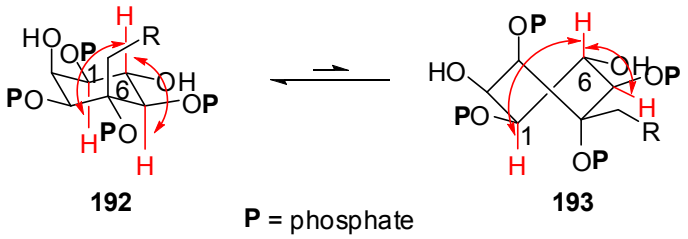


Figure 5.9: The primary 4-*C*-alkyl derivatives occupy the natural chair conformation

If the 4-*C*-alkyl group was occupying a pseudo equatorial position in a twist-boat conformation in order to relieve 1,3-diaxial strain, the dihedral angle between the protons adjacent to the 6-*H* would be reduced, and this would be visible in the size of the coupling constants. The coupling constants with the larger 4-*C*-alkyl bumps were reduced slightly, suggesting greater occupancy of a twist-boat conformer. The natural chair conformation is essential for binding to the modified proteins in RLE. If the 4-*C* analogues were locked in another conformation they would be unable to fit into the highly conserved binding site.

The identity of these compounds was confirmed by HRMS in negative ion mode. The fully deprotected polyphosphates bind tightly to any metal ions and successful ionisation was only obtained using MeCN-H_2O (1:1) with 1 % formic acid. The presence of TFA due to contamination of service instruments caused initial difficulties as TFA clusters completely supressed analyte peaks.

The 4-*C*-methyl, -ethyl and -pentyl triols **175a-c** were phosphitylated in a similar manner to the tetrols, using **93b**, followed by oxidation using *m*CPBA to give the corresponding 1,4,5-triphosphates **194a-c**, Figure 5.10.

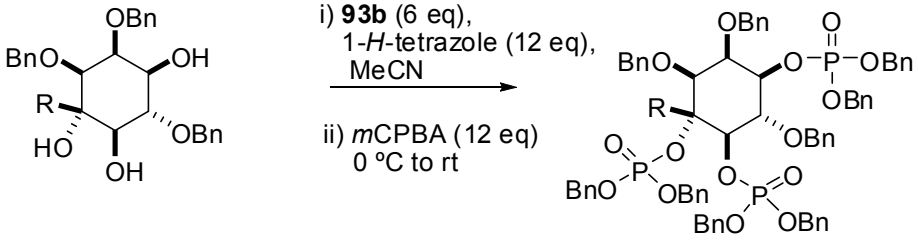


Figure 5.10: Phosphorylation of 4-*C*-methyl, -ethyl and -pentyl triols

In each case ^{31}P-NMR confirmed that phosphorylation had occurred on three sites. For the 4-*C*-methyl IP_3 **194a**, the ^{1}H and ^{13}C-NMR spectra were sharp, but with increasing alkyl chain

length the inositol resonances became increasingly broad. Oxidation of the P(III) phosphite triesters has been reported using both *m*CPBA and ^{t}BuOOH.[110,125] Yu and Reid[110] noted that oxidation with *m*CPBA is cleaner than the corresponding oxidation with ^{t}BuOOH. Oxidation of the intermediate 4-*C*-methyl 1,4,5-triphosphite was attempted with both oxidising agents and the crude ^{31}P-NMR compared, Figure 5.11.

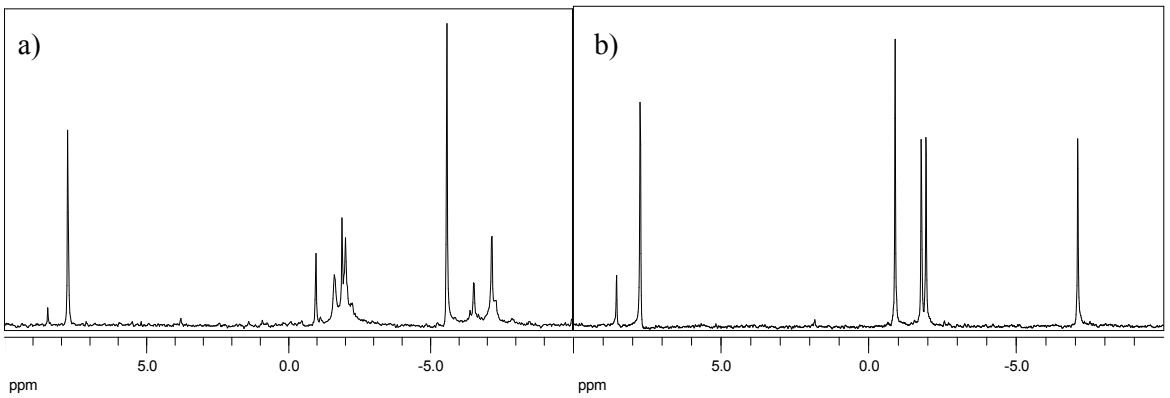
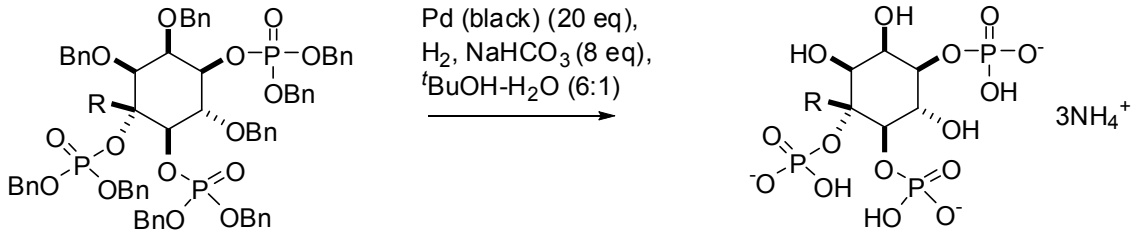


Figure 5.11: Comparison of ^{1}H-NMR of the crude material resulting from oxidation with either a) ^{t}BuOOH or b) *m*CPBA.

The ^{1}H-NMR of the crude material resulting from oxidation with ^{t}BuOOH clearly shows a number of peaks resulting from impurities, compared to the oxidation with *m*CPBA which only shows the three product peaks and reagent debris. Following the incomplete phosphorylation of the branched or benzyl 4-*C*-alkyl tetrols with **93b**, phosphorylation of the corresponding triols was not attempted.

Deprotection of the nine benzyl groups with Pd (black) under an atmosphere of H_2 afforded 4-*C*-methyl, -ethyl and -pentyl IP_3, Figure 5.12.



194a R = Methyl
194b R = Ethyl
194c R = Pentyl

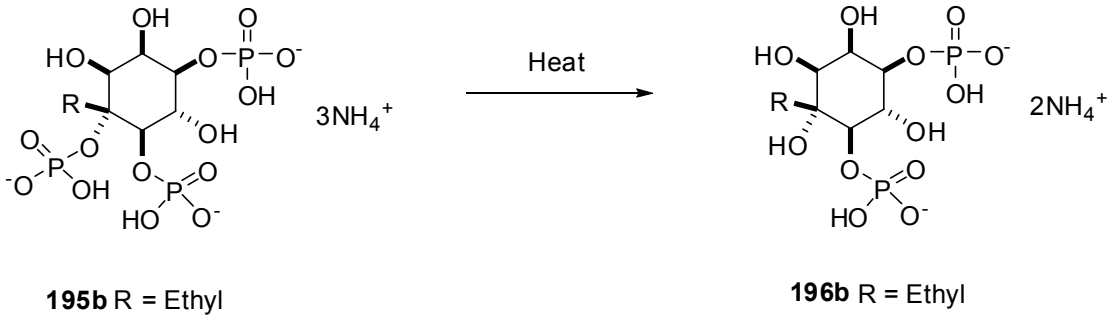
195a R = Methyl, [100 %]
195b R = Ethyl, [100 %]
195c R = Pentyl, [100 %]

Figure 5.12: Global deprotection of benzyl groups to generate 4-*C*-methyl, -ethyl and -pentyl IP_3

In each case all the benzyl groups were successfully removed (no signals δ_H 7-8 ppm). The 4-*C*-methyl IP_3 was a single product with sharp ^{1}H-NMR signals at rt. Like the corresponding 4-*C*-methyl IP_4, the 6-*H* coupling constants were typical of having two adjacent anti-periplanar protons

[δ_H 3.75 (1H, dd, *J* 9.9, 9.8 Hz) ppm] and there was a clear NOESY between the 4-*C*-methyl (1.48 ppm) and the 6-*H* corroborating the required stereochemistry was present at the 4-*C*.

For both the 4-*C*-ethyl and -pentyl IP_3 there was evidence in the ^{1}H-NMR that another inositol phosphate product was present. Presumably as a result of having one less equatorial phosphate, these 4-*C*-alkyl IP_3 were notably more fluxional than the corresponding 4-*C*-alkyl IP_4's. The spectra at rt contained very broad signals corresponding to the 4-*C*-alkyl IP_3 and sharper peaks from an unknown inpurity. Variable temperature NMR was carried out in order to identify the impurity and more firmly characterise the final product. For the 4-*C*-ethyl IP_3, resolution of the product peaks was much improved as the temperature reached 328 K. Unfortunately, as the sample was held at this temperature the product decomposed, turning entirely into the impurity. This permitted identification of the impurity as 4-*C*-ethyl-*myo*-inositol-1,5-diphosphate, resulting from dephosphorylation of the 4-*O*, Figure 5.13.



195b R = Ethyl

196b R = Ethyl

Figure 5.13: 4-*C*-ethyl-*myo*-inositol-1,5-diphosphate

Although the hydrogenation was buffered using $NaHCO_3$, some initial dephosphorylation may have occurred during purification by ion exchange, where the crude material was converted to the free acid to remove all the counter ions. The free acid was neutralised by addition of aqueous ammonia, chosen due to its volatility, as excess NH_3 could be removed under vacuum. However, upon heating, the weakly acidic NH_4^+ counter ions may protonate the tertiary phosphate ester resulting in the observed 4-*O*-dephosphorylation. Although in isolated phosphate esters this would not happen, in inositol polyphosphates neighbouring phosphates effectively raise each others pKa due to charge repulsion.

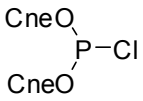
Further samples of 4-*C*-ethyl and -pentyl IP_3 were prepared and, in order to avoid acidic conditions, stored as the sodium salt. After hydrogenation these compounds were stirred with sodiated ion exchange resin to remove any remaining Pd^{2+} and to avoid preparation of the free acid. Unfortunately the sodium salts were difficult to analyse due to excess sodium bicarbonate present in the sample (δ_H 8.36, δ_C 161.14 ppm). The ^{1}H-NMR signals of both compounds were still broad, and the ^{31}P-NMR even broader than previously encountered. Furthermore, it was evident that even after multiple hydrogenations, a set of aromatic peaks [δ_H 7.74 (2H, d), 7.41 (1H, t), 7.35 (2H, t)] was present, either from incomplete hydrogenation or an aromatic contaminant. The MS of these

samples also suffered from the presence of excess sodium ions and from multiple sodiated clusters as some Na^+ ions were ionised with the sample. Therefore it was not possible to confirm whether these peaks were due to only partial hydrogenation of the 2- and 6-*O*-benzyl groups, or due to presence of another species e.g. benzoic acid, from an unknown source.

4-*C*-Ethyl IP_3 was therefore fractionated by HPLC, eluting with MeCN-H_2O (1:49 v/v), to isolate the pure product. Unfortunately, the contaminant was not isolated, and therefore could not be identified.

5.2 More reactive phosphorylating reagents for bulky 4-*C*-alkyl substituents

Having successfully achieved phosphorylation and deprotection of the straight chain 4-*C*-alkyl triols and tetrols, other phosphitylating reagents were considered for use on larger 4-*C*-alkyl substituents, such as the 4-*C*-benzyl derivative. Following incomplete phosphitylation of the 4-*C*-benzyl 4-*O*H with phosphoramidite **93b**, it was hoped that a more reactive phosphitylating reagent would effect complete phosphorylation of the tertiary 4-*O*H. Di(2-cyanoethyl) phosphorochloridite **91** was available in the lab and has been found by the group to be a highly reactive polyphosphitylating reagent, Figure 5.14.



91 Cne = $-CH_2CH_2CN$

Figure 5.14: Dicyanoethyl phosphorochloridite

Prepared by the reaction of trimethylsilyl(3-propionitrile) with PCl_3, this reagent can be stored under N_2 as a solution in CH_2Cl_2.[113] Pleasingly, phosphitylation of 4-*C*-benzyl tetrol **151f** with **91**, in the presence of 3-nitro-1,2,4-1*H*-triazole, followed by oxidation of the resulting tetraphosphite by *m*CPBA prepared the desired tetraphosphate, with no evidence of triphosphate due to incomplete phosphorylation, Figure 5.15.

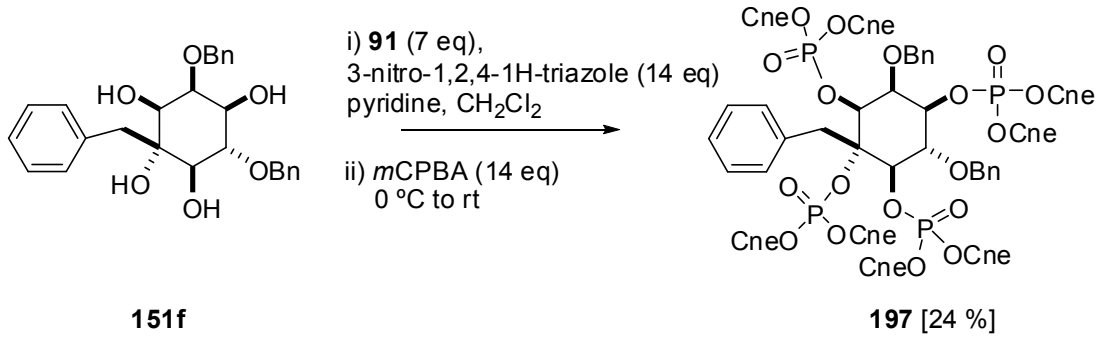


Figure 5.15: Phosphorylation of 4-*C*-benzyl tetrol with **91** prepares the 1,3,4,5-tetraphosphate

The phosphorylation was carried out in pyridine to neutralise the HCl evolved during the reaction. Due to the known presence of a small amount of the dichloridite in this reagent,[113] cyanoethanol was added to the reaction 30 min prior to the oxidation. The low yield of product recovered, 24 %, reflects the difficulty in visualising this fully protected species. Despite using many different TLC dips and stains, the most successful method of visualisation was $KMnO_4$ stain, but this was still insensitive.

The cyanoethyl phosphate esters and 2,6-*O*-benzyl ethers on **197** require two separate steps to effect total deprotection. In order to prevent migration of phosphotriesters, the cyanoethyl protected phosphate esters must first be deprotected, followed by the removal of the 2- and 6-*O* benzyl groups. Treatment of **197** with Barton's base, in the presence of TmsCl, generated an intermediate tetra[bis(trimethylsilyl)phosphate].[113] After evaporating the volatile material, including acrylonitrile derived from the cyanoethyl esters and excess Bartons's base, the residue was taken up in ether-hexane (1:1 v/v) and filtered under anhydrous conditions to remove excess Barton's base salts. The filtrate was evaporated and taken up in MeOH containing 4 eq. $NaHCO_3$ to convert the phosphoric acid groups to their mono-sodium salts as they formed, Figure 5.16.

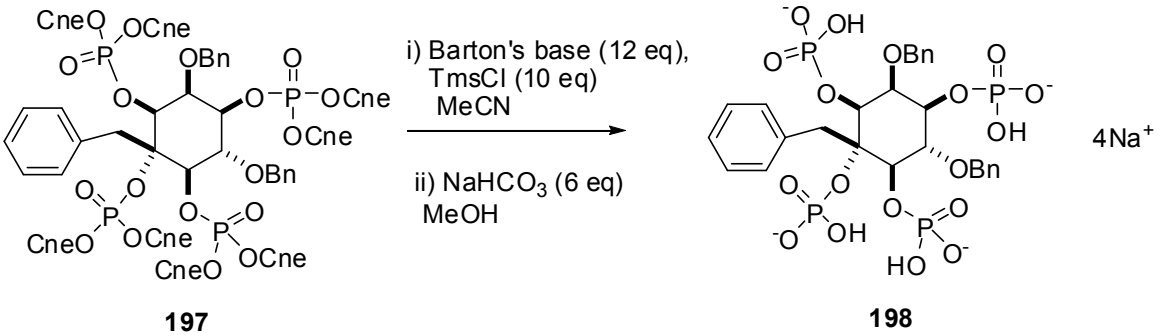


Figure 5.16: Removal of cyanoethyl groups to generate free phosphates and conversion to the sodium salt

Well resolved ^{1}H-NMR clearly showed the aromatic protons of three phenyl groups; the 2- and 6-*O*-benzyl ethers and 4-*C*-benzyl substituent, and the mass was confirmed by HRMS. Despite care during the filtration, ^{13}C and ^{1}H-NMR showed evidence of some Barton's base counter ions. However it was anticipated that this could be removed during later cation exchange.

2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol-1,3,4,5-tetraphosphate **198** was treated with Pd (black) under an atmosphere of H_2. After 36 h, the crude material was treated with sodiated ion-exchange resin. However, ^{1}H-NMR indicated that deprotection was not complete; more than one phenyl group was still visible in the aromatic region and at least two sets of 4-*C*-benzyl peaks were visible. After a further 36 h under the same conditions, and ion exchange in the same manner, it was evident that the deprotection was nearer to completion; distinctive peaks of the single remaining 4-*C*-aromatic ring were visible [δ_H 7.38 (2H, d, *J* 8.0), 7.18 (2H, t, *J* 7.6), (1H, t, *J* 7.2) ppm]. However, there were still trace amounts of other aromatic compounds, confirmed by HRMS to be the partially deprotected mono-benzyl species [HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 590.9850 (17), $C_{13}H_{23}O_{18}P_4$ requires 590.9835 and $[M+Bn+H]^+$ 681.0322 (33), $C_{20}H_{29}O_{18}P_4$ requires 681.0304]. Furthermore, new peaks were observed in the 2-0.8 ppm region of the ^{1}H-NMR, suggesting that not only was deprotection incomplete, but that competing hydrogenation of the 4-*C*-benzyl group to a 4-*C*-(cyclohexyl)methyl substituent was occurring.

Unfortunately, the small amounts of material available from this route did not permit isolation of the desired compound, hampered as it was by the competing hydrogenation of the 4-*C*-benzyl group.

5.3 Phosphorylation with less hindered *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane

Successful phosphorylation of 4-*C*-benzyl tetrol with di(2-cyanoethyl)phosphorochloridite, **91**, provided evidence that it was possible to effect complete phosphorylation at this tertiary 4-*O*H. Unfortunately, clean deprotection of 1,3,4,5-*O*-tetrakis(dicyanoethyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol **197** was not possible. Consequently, a reagent was sought that was more reactive than dibenzylphosphoramidite **93b**, but that would still permit a one step deprotection by hydrogenation. Therefore, *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane was prepared, according to the methods of Arbuzov.[121] Watanabe *et al.*[111] report that this more compact reagent was particularly useful for polyphosphorylation of multiple sterically crowded sites. Arbuzov *et al.*[121] report the preparation of both the *N,N*-dimethyl- and -diethylaminophosphepane, which can be purified by distillation. The ethyl reagent, **94b**, was prepared in the hope that this derivative would be somewhat more stable to hydrolysis than the corresponding dimethyl reagent, and therefore would be easier to handle and store, Figure 5.17.

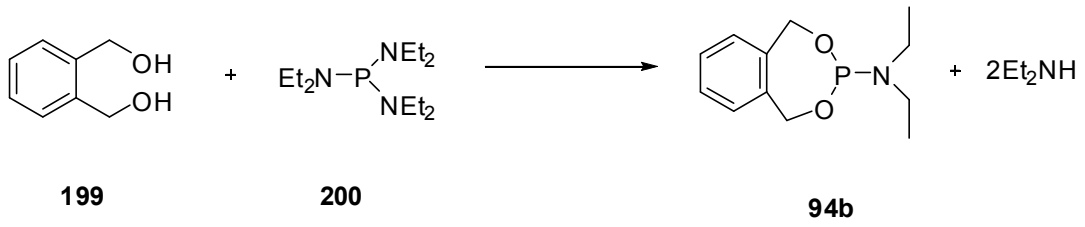


Figure 5.17: Preparation of *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane

A test reaction on the already successfully phosphorylated 4-*C*-methyl tetrol **151a** demonstrated that the reagent gave very clean ^{1}H- and ^{31}P-NMR spectra of the crude product, with reagent peaks at δ_P 13.16 and 12.51 ppm. However, purification by column chromatography failed to separate the product from the reagent debris. On initial phosphitylation of 4-*C*-benzyl 2,3,6-*O*-tribenzyl ether **175f** with **94b**, followed by oxidation by *m*CPBA, a mixture of bis- and trisphosphorylated species **201** and **202** was obtained in the ratio 9:13, visible as one spot by TLC, Figure 5.18.

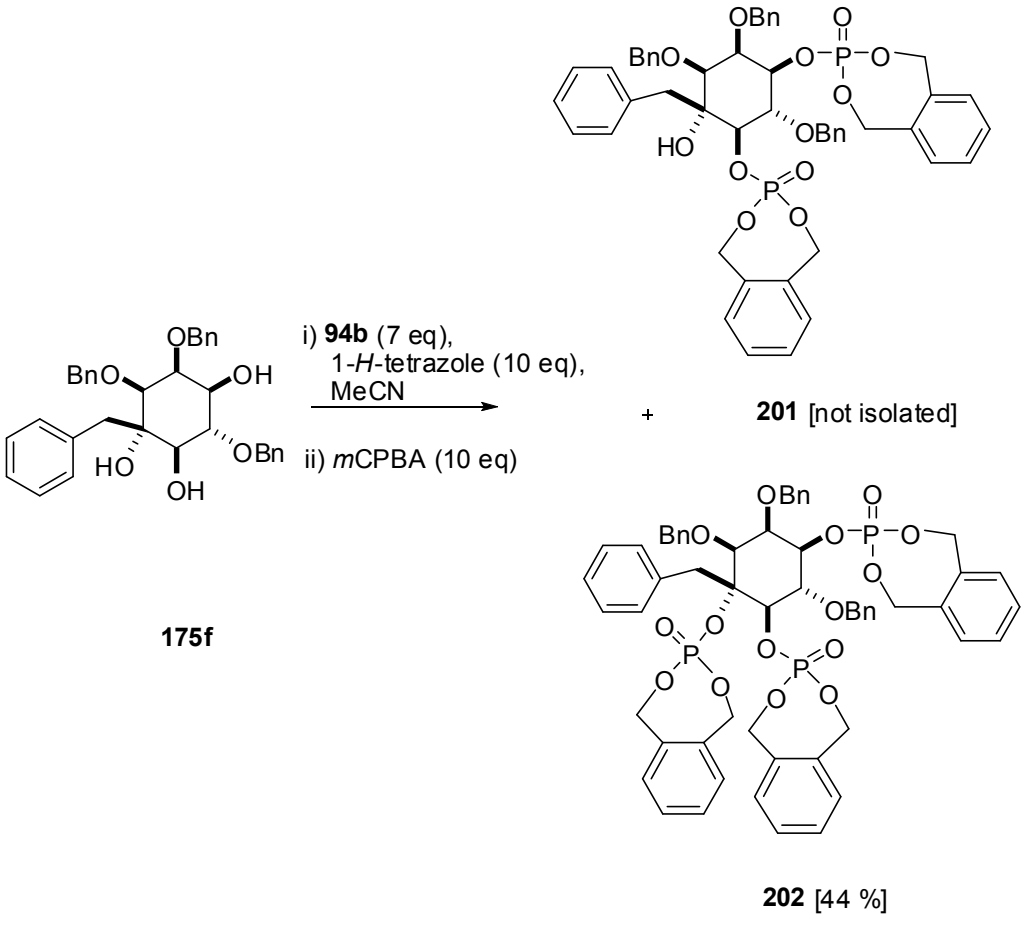


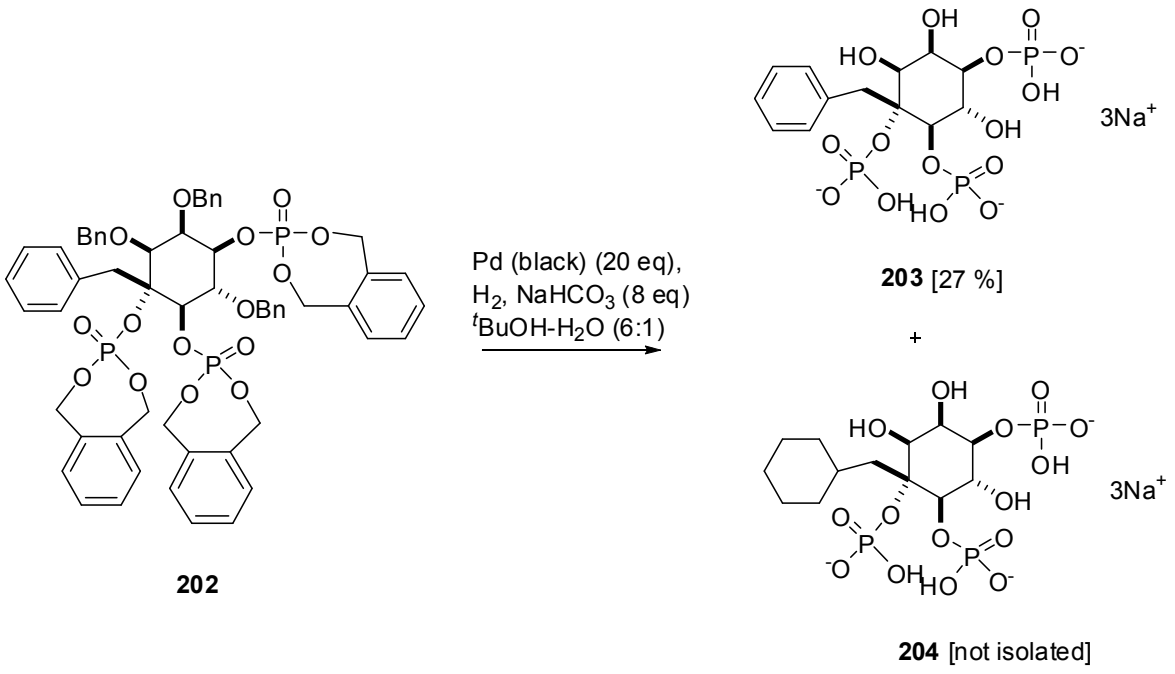
Figure 5.18: Phosphorylation of 4-*C*-benzyl triol using **94b**.

Separation of a small amount of diphosphate from the mixture confirmed its identity [HRMS *m/z* (%) found $[M+H]^+$ 905.2889 (63), $C_{50}H_{51}O_{12}P_2$ requires 905.2856, $[M+Na]^+$ 927.2714 (100), δ_P 0.64, -1.25 ppm]. By maintaining the temperature of the reaction at 25 ºC using a water

bath, and increasing the concentration of the reaction mixture by halving the amount of solvent used, it was possible to observe complete phosphorylation on all three hydroxyls, generating the desired triphosphate, **202**, not contaminated with diphosphate **201**. Unlike with the earlier *N,N*-diisopropyl phosphoramidite, the diethyl reagent did not suffer from thick precipitation during the reaction, and therefore it was possible to increase the reaction concentration and maintain stirring. Purification of the crude material was hampered by difficulty in visualising the product, and the substantial amount of phosphorylating reagent debris remaining, which had the same R_f as the product. Even so, use of a slow gradient column successfully isolated triphosphate **202** in 44 % yield.

Following this success, phosphorylation of 4-*C*-benzyl tetrol **151f** and 4-*C*-neopentyl tetrol **151e** were attempted. In both cases the crude ^{1}H-NMR was not as clean as that of the 4-*C*-benzyl triol. Upon purification of the 4-*C*-neopentyl tetraphosphate, some triphosphate was recovered. The absence of a downfield ^{31}P-NMR peak in addition to three ^{31}P-NMR peaks around 0 ppm confirmed that this was due to lack of phosphorylation of the tertiary 4-*O*H. For the 4-*C*-benzyl tetraphosphate, HRMS of the crude material indicated that there was no triphosphate present and that the desired tetraphosphate was present, but this was not isolated from the remaining phosphorylating reagent debris.

1,4,5-*O*-Tris(5,6-benzo-1,3,2-dioxaphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol, **202**, was globally deprotected with Pd(black) under an atmosphere of H_2, Figure 5.19.



202

203 [27 %]

+

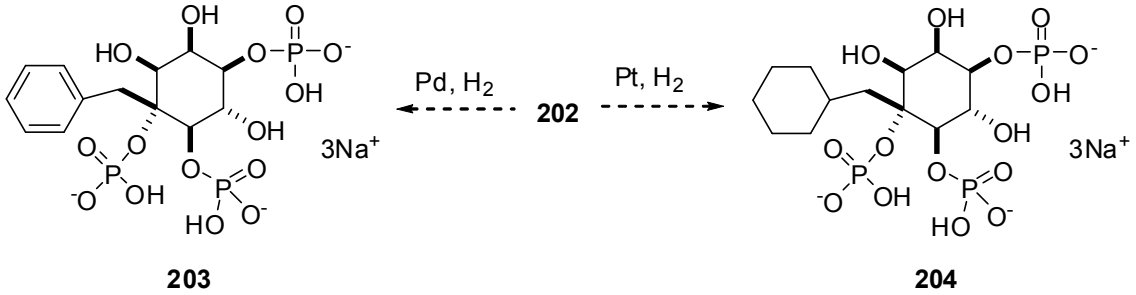
204 [not isolated]

Figure 5.19: Global deprotection of **202**

After stirring with sodiated ion exchange resin, the ^{1}H-NMR of the crude material clearly showed 5 aromatic protons [δ_H 7.44 (2H, d, *J* 7.5), 7.22 (2H, t, *J* 7.4), 7.14 (1H, t, *J* 7.3) ppm], confirming that full 2-, 3- and 6-*O*-benzyl deprotection had occurred. However, there was also evidence of over-reduction of the 4-*C*-benzyl group to the 4-*C*-(cyclohexyl)methyl derivative with several peaks, in the 2-0.8 ppm region of the ^{1}H-NMR. The ratio of the two products was estimated to be 4:1 in favour of the desired 4-*C*-benzyl product. However separating these very similar compounds was not easy. The desired final product **203** was isolated using HPLC (retention time 2.88 min, mobile phase H_2O-MeCN gradient (49:1 → 1:1 v/v). However a large amount of the injected material was not recovered, possibly due to chelation to the exposed steel interior surfaces the HPLC equipment.[148]

Due to the highly similar and polar nature of these products, it is not possible to monitor the reaction by TLC, and therefore it would be preferable to develop methods which selectively reduce only the desired functional groups. Pearlman's catalyst[149] has been used to selectively remove benzyl ethers in the presence of phenyl rings.[150,151] Reduction of **202** with Pearlmans catalyst under an atmosphere of H_2 saw only partial deprotection of the benzyl groups. There was no evidence of over reduction, although the reaction had only gone to ~45 % in 24 h, so it is possible that over-reduction would be observed on the timescale required to effect complete deprotection.

For any 4-*C*-derivatives which are unsaturated, such as the 4-*C*-benzyl or -(2-naphthyl)methyl analogues, careful control of the hydrogenation could be used to generate more than one 4-*C*-derivative. For example, the 4-*C*-benzyl substituent could be reduced to the 4-*C*-(cyclohexyl)methyl group during deprotection of the benzyl ethers, Figure 5.20. The key challenge would be to either develop selective deprotection conditions for the 2- and 6-*O* benzyl ethers without simultaneous hydrogenation of the 4-*C*-benzyl substituent, or be able to effect complete hydrogenation, including the reduction of the benzyl to the cyclohexyl. The selective removal of benzyl ethers in the presence of other sensitive groups such as aryl chlorides and bromides is described using triethylsilane catalysed by palladium acetate.[151] Theoretically, both species may be accessed during this last step of the synthetic route, making this an attractive route to further 4-*C*-alkyl substituents.



203 **204**

Figure 5.20: The 4-*C*-benzyl group may be selectively reduced to generate both the 4-*C*-benzyl and the 4-*C*-(cyclohexyl)methyl IP_3 products

Since 4-*C*-(cyclohexyl)methyl IP_3 is a viable 4-*C*-alkyl target in its own right, complete reduction of **202** was attempted using Raney nickel. Unfortuately this showed no further reaction on the mixture of products compared to treatment with Pd(black). It is postulated that as the benzyl groups are removed, the increasingly polar species have a decreasing affinity for the solid phase catalyst, compared to the hydrophobic phenyl ring of the *C*-benzyl group, and this may lead to the lack of selectivity observed in this system compared to literature reports which indicate these catalysts are selective.[152] If this is the case, then a homogeneous catalyst may provide the selectivity needed by overcoming the problems associated with restricted access to the benzyl ethers for a solid catalyst face.

5.4 Concluding Remarks

It has been demonstrated in this chapter that phosphorylation of a 4-*C*-alkyl tertiary centre is possible. For less sterically demanding straight chain *C*-alkyl groups this was possible using *N,N*-diisopropyldibenzyl phosphoramidite which generated 4-*C*-alkyl IP_3 and IP_4 precursors with nine or ten benzyl groups respectively. These globally protected precursors could be fully deprotected with Pd(black) under an atmosphere of H_2. Following the obervation of 4-*O*-dephosphorylation of their ammonium salts, these final 4-*C*-alkyl products were stored as the more stable sodium salts. For larger 4-*C*-alkyl groups, the less sterically demanding *N,N*-diethyl cyclic phosphoramidite was successfully employed to prepare fully protected 4-*C*-benzyl IP_3. During subsequent total deprotection this derivative proved easier to unblock than attempts to deprotect a related cyanethyl protected phosphate, followed by removal of the benzyl ethers. The use of the cyclic phosphoramidite for the phosphorylation of 4-*C*-benzyl tetrol and 4-*C*-neopentyl tetrol seems promising, although more time is needed to optimise the purification of these fully protected derivatives.

5.5 Biological Results

5.5.1 4-*C*-methyl IP_3 as a substrate for IP_3Rs

Inositol triphosphate receptors (IP_3R)s undergo a conformational change upon binding of IP_3,[153] which is thought to result in opening of the ion channel, allowing release of Ca^{2+}. It was hoped that a small 4-*C*-alkyl group would cause a steric clash which would prevent conformational change in the tight binding site, thereby acting as an antagonist. 4-*C*-Methyl IP_3, **195a**, was tested by Geert Bultynck who performed *in vitro* assays to compare it to natural IP_3 as a ligand for IP_3Rs,[154] and therefore determine its ability to release Ca^{2+}. 4-*C*-Methyl IP_3 did release Ca^{2+} from permeablised L15 cells, and a dose-response curve was measured, Figure 5.21.

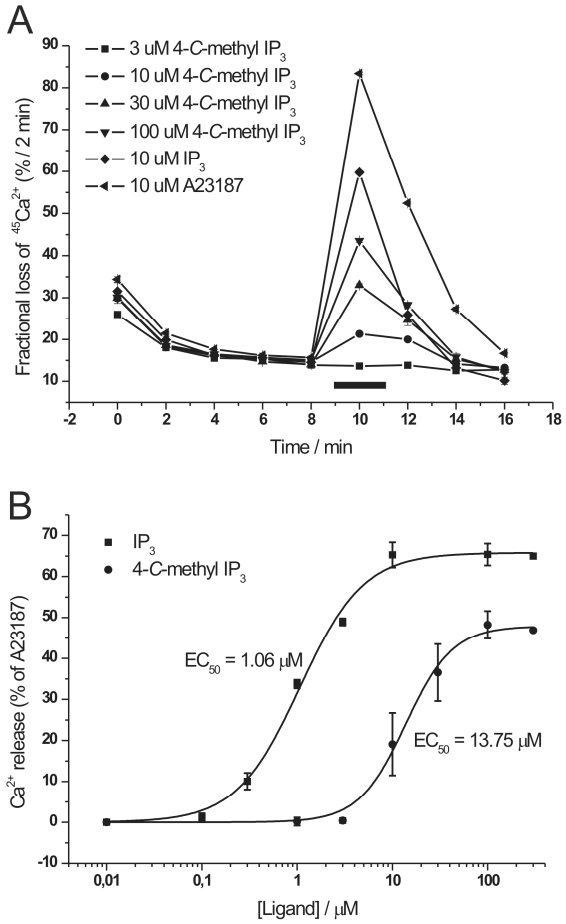


Figure 5.21: Experiments carried out by Geert Bultynck: A) A typical experiment in permeabilised L15 cells showing the fractional loss of $^{45}Ca^{2+}$ with time. The black bar indicates the addition of IP_3 or 4-*C*-methyl IP_3 (**195a**). Cells were treated with A23187 to estimate the maximal releasable $^{45}Ca^{2+}$; B) Dose response for IP_3R-dependent Ca^{2+} release provoked by IP_3 or 4-*C*-methyl IP_3 (**195a**) in permeabilised L15 cells. Values were normalized to the A23187-releasable Ca^{2+}. Data points were obtained from at least 3 independent experiments and are plotted as mean +/- standard error of the mean. EC_{50} values were obtained by sigmoidal curve fitting (Origin® 7.0).

The EC_{50} of 4-*C*-ethyl IP_3 was 13.75 μM, compared to 1.06 μM for IP_3. Only 45 % of the available Ca^{2+} was released (calculated by comparision with the Ca^{2+} released by ionophore A23187). This result was encouraging: 4-*C*-methyl IP_3 was able to release Ca^{2+}, suggesting that it bound to the IP_3R, and therefore confirming that the natural chair conformation is occupied. The release of a lesser percentage of the available Ca^{2+} than natural IP_3 may also suggest that partial inhibition of these receptors is being observed, or that 4-*C*-methyl IP_3 is selective for one of the three IP_3R subtypes.

Larger 4-*C*-alkyl groups will be tested using this assay in the future, and it is hoped that they may provide interesting results for probing this pathway.

5.5.2 4-*C*-Methyl IP_3, 4-*C*-methyl, -ethyl and -pentyl IP_4 as substrates and inhibitors of synaptojanin

The type II phosphatase synaptojanin can hydrolyse PtdIns(4,5)P_2, PtdIns(3,4,5)P_3, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 and has attracted particular interest due to its possession of two phosphatase domains in one protein.[155] Synaptojanin has both a central inositol 5-phosphatase domain and an *N*-terminal Sac1 homology domain, which hydrolyses the 3, 4 and 5-monophosphates and PtdIns(3,5)P_2. Therefore it has the potential to generate PtdIns without the production of any intermediate monophosphates.

4-*C*-Methyl IP_3 (**195a**), 4-*C*-methyl (**191a**), -ethyl (**191b**) and -pentyl IP_4 (**191c)** were tested by Jessica Knott as substrates, competitive and uncompetitive/allosteric inhibitors of synaptojanin. The four compounds were tested as substrates by direct addition to the synaptojanin construct, followed by addition of malachite green to determine the amount of free phosphate generated. Unfortunately 4-*C*-methyl and -pentyl IP_4 **191a** and **c** appeared to contain too much free phosphate in the sample, and therefore were not measurable within the range of the assay. Of the remaining 4-*C*-methyl IP_3 and 4-*C*-ethyl IP_4, neither compound was a substrate for the 5-phosphatase domain, even at a high concentrations (400 μM), Figure 5.22

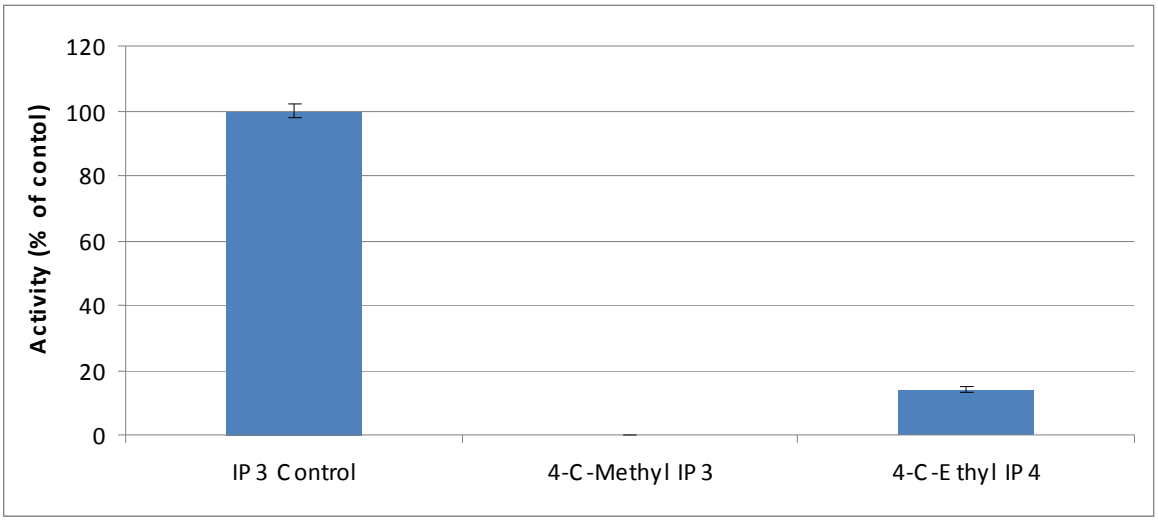


Figure 5.22: Testing 4-*C*-methyl IP_3 and 4-*C*-ethyl IP_4 as a substrate for synaptojanin

In the inhibition assay, the hydrolysis of natural IP_3 by synaptojanin was reduced by 50 % with a substrate concentration of K_M, and by 25 % with a substrate concentration of V_{max}, by 4-*C*-ethyl IP_4 **191b** which appeared to be acting as a competitive inhibitor, Figure 5.23.

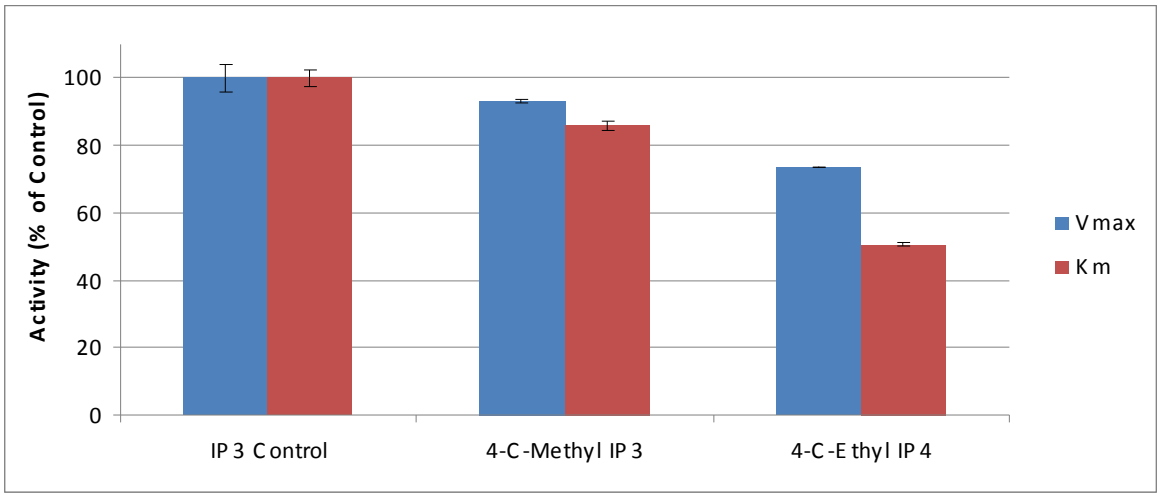


Figure 5.23: Testing the inhibition of synaptojanin with 4-*C*-methyl IP_3 and 4-*C*-ethyl IP_4

Therefore a dose response curve for 4-*C*-ethyl IP_4 was measured which demonstrated that further increasing its concentration did not increase the inhibition of IP_3 hydrolysis, Figure 5.24

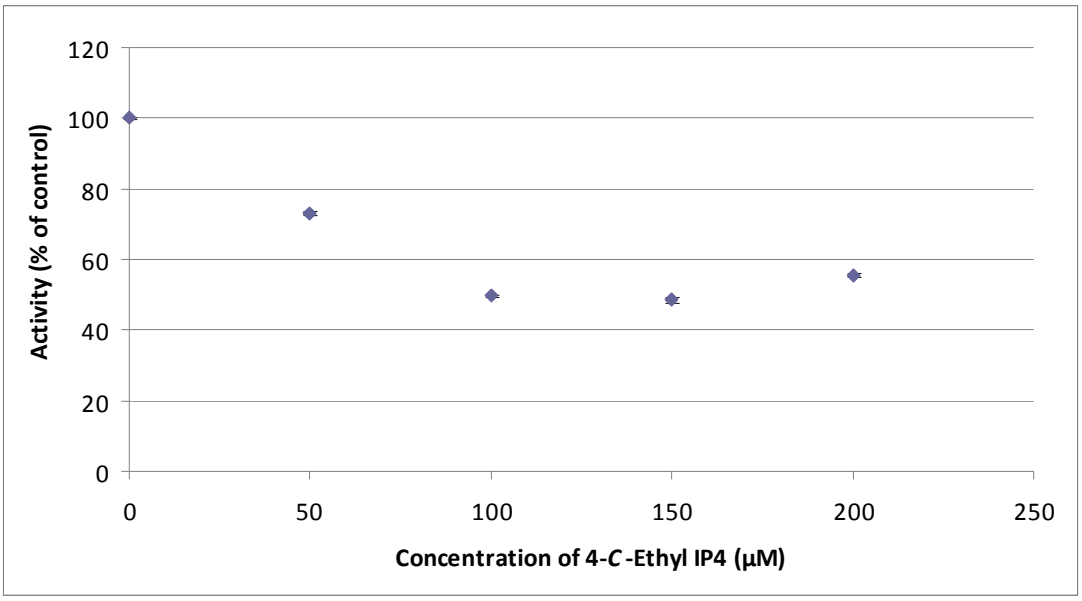


Figure 5.24: Dose-response curve for 4-*C*-ethyl IP_4

The dual-phosphatase domains of Synaptojanin make it complex to decipher its mechanisms of action and to study the effects of the individual domains in isolation. Therefore selective inhibitors of one phosphatase domain are valuable. The 4-*C*-ethyl IP_4 was assayed with 5-phosphatase dead and Sac dead mutants to establish if it was a specific inhibitor of just one of the phosphatase domains. The 5-phosphatase dead mutant showed slightly higher activity than the control, suggesting that this phosphatase domain was not inhibited. The Sac dead mutant showed 50 % inhibition in the presence of 4-*C*-ethyl IP_4 inhibitor, Figure 5.25.

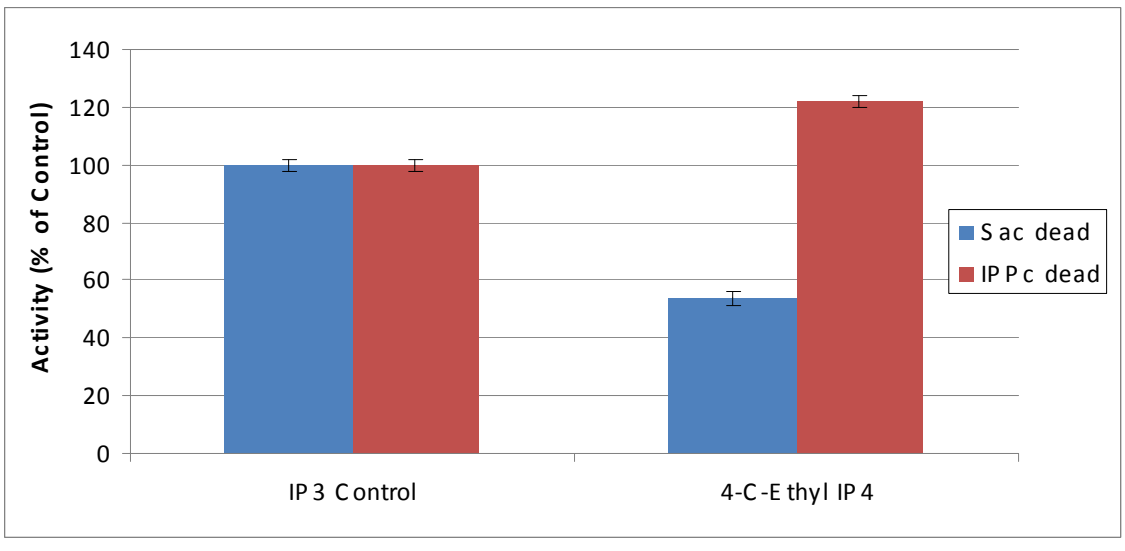


Figure 5.25: Testing the inhibition of 4-*C*-ethyl IP_4 on 5-phosphatase and Sac dead mutants

Both experiments were carried out at V_{max} concentrations of IP_3. This result suggests that 4-*C*-ethyl IP_4 may be a selective inhibitor of the synaptojanin 5-phosphatase domain. This

compound may therefore be useful in the elucidation of the function of the Sac domain. The results for the inhibition of the two isolated domains at $[IP_3] = K_M$ are still pending.

For the RLE project, it is not desired that the 4-*C*-alkyl IP_n are turned over metabolically or act as competitive inhibitors of natural cellular proteins. It is hoped that they will only interact with their complementary modified binding proteins. The inhibition observed with synaptojanin only occurred at relatively high (μL) concentrations of 4-*C*-ethyl IP_4 analogue **191b**. This suggests that as the size of the *C*-alkyl group increases, the modified ligand should become orthogonal to synaptojanin, and other natural proteins.

Chapter 6: 4-, 5- or 6-*C*-alkyl substituents from a common intermediate

Chapters three to five of this thesis have focussed on the introduction of a 4-*C*-alkyl substituent and the subsequent protecting group manipulations, phosphorylation and deprotection required to generate a 4-*C*-alkyl inositol phosphate head group. This position was chosen to allow rapid access to an isolated hydroxyl for manipulation. The inositol head-group binds with the inositol ring roughly parallel to the protein surface, suggesting that any one of the 1-, 3-, 4-, 5- or 6-*H* may point towards the binding pocket during binding to different types of receptor (see Figure 1.13). Ultimately, any one of these protons might need to be substituted by a *C*-alkyl group for a particular application. Therefore, during the development of the 4-*C*-alkyl synthesis, it became apparent that a more divergent synthesis which permitted access to more than one ring position would be of great benefit.

At first glance, this aim appears to require the sequential isolation of each hydroxyl, in order to introduce an alkyl group at that position. Of the six inositol hydroxyls the 5-*O*H is the most difficult to isolate. This is substantially due to its distance from the axial 2-*O*H, which directly modulates the reactivities of the neighbouring hydroxyls, but also distinguishes one face of the inositol ring from the other. Therefore it may be anticipated that introducing a 5-*C*-alkyl would be particularly challenging. It was postulated that by manipulation of the inositol hydroxyls' stereochemistry, a *C*-alkyl group introduced at one location could effectively be manoeuvred around the ring by coordinated hydroxyl inversions to generate multiple *C*-alkyl groups from a common intermediate.

This chapter is divided into three sections. First, an introduction to the new strategy for generating 4-, 5- and 6-*C*-alkyl substituents, including a review of the literature which has led to this proposal. Secondly, the results from a test system and initial application of this approach, generating a 2-*C*-alkyl group and further manipulations, are reported. The final section looks at the prospects for this strategy and future work.

6.1 A general method for the preparation of 4-, 5- and 6-*C*-alkyl substituents

myo-Inositol has one axial hydroxyl. If this hydroxyl is converted to an equatorial orientation, a *scyllo*-inositol framework is generated, with all 6 hydroxyls in an equatorial orientation. If this is followed by one further inversion of a different equatorial hydroxyls'

stereochemistry (potentially at any one of the ring carbons) the *myo*-geometry is regenerated but with the reference frame rotated, Figure 6.1.

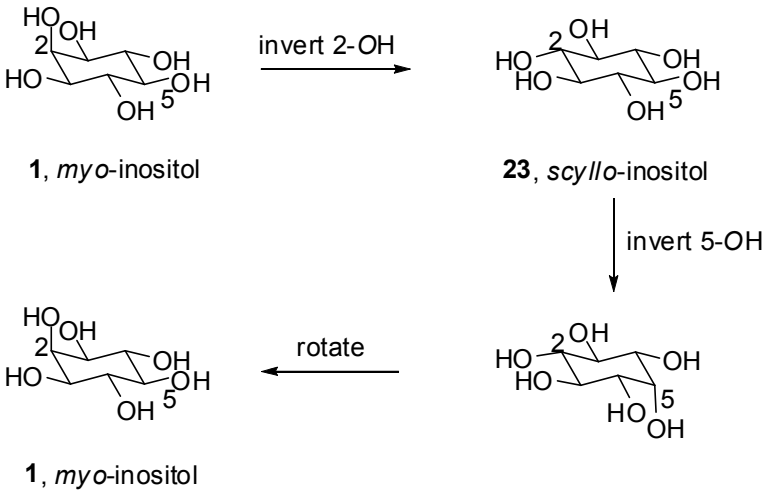


Figure 6.1: Manipulation of the ring geometry via a *scyllo*-inositol by hydroxyl inversion and subsequent reintroduction of *myo*-geometry.

Inversion of a hydroxyl could be carried out by oxidation of one ring position to the inosose, followed by stereospecific reduction. The stereoselective conversion of a *myo*-inositol framework to *scyllo*-derivatives has been reported for inositol orthobenzoates, Figure 6.2.

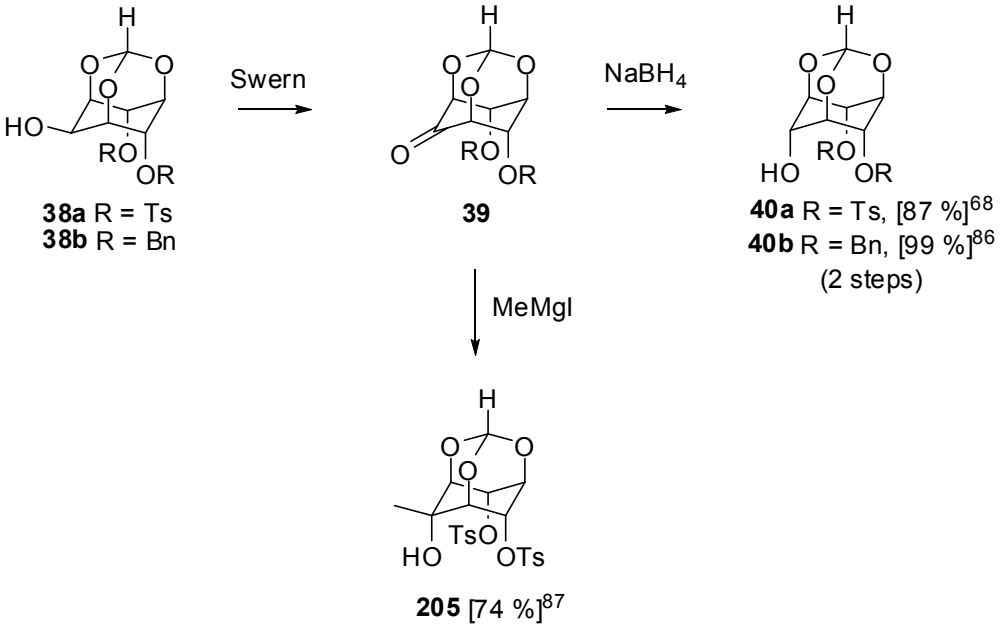


Figure 6.2: Reduction of inos-2-ose orthoester to generate *scyllo*-geometry products.

Oxidation of the 2-*O*H to the inos-2-ose is widely reported[70,82,88,89] (see chapter 2.3). Both selective reduction with $NaBH_4$,[70,89] and addition of MeMgI are reported[90] to generate protected *scyllo*-inositols. Both hydride and methyl delivery occurred stereoselectively to the top face of the

inososе, via an axial approach to the carbonyl. The reintroduced hydroxyl is axial (N.B. equatorial once the orthoester is removed) corresponding to a *scyllo*-geometry framework.

It has been demonstrated in chapter 3 that a wide variety of 4-*C*-alkyl substituents can be introduced by nucleophilic opening of the *exo*-methylene oxide. Consequently, preparation of the inos-2-ose, conversion to the *exo*-methylene oxide, followed by ring opening with lithium alkyl cuprates, should permit access to the same range of 2-*C*-alkyl derivatives. The proposed series of 2-*C*-alkyl intermediates would be of *scyllo*-geometry resulting from delivery of methylene to the top face of the inososе, in the preparation of the *exo*-methylene oxide **207**, Figure 6.3.

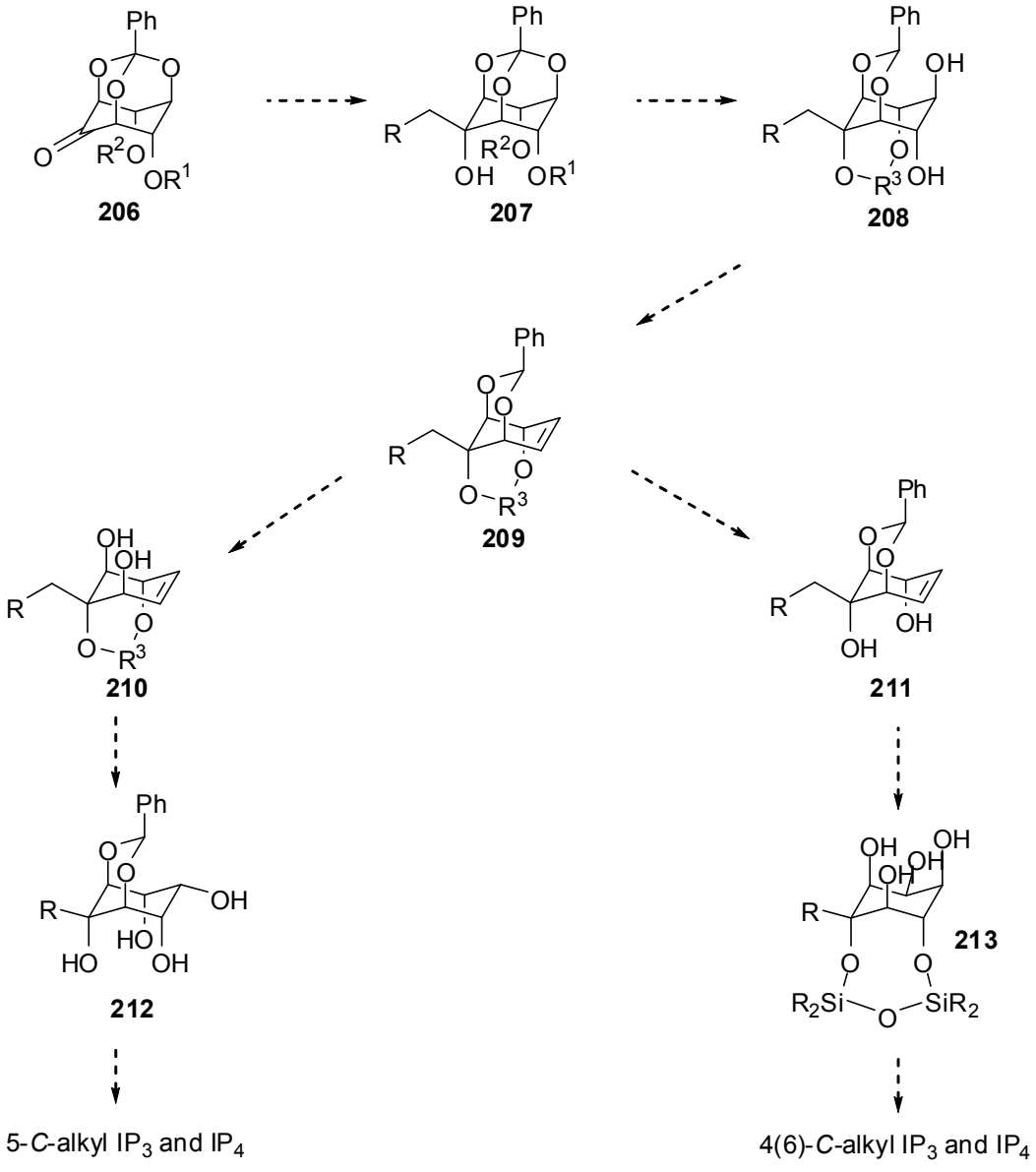


Figure 6.3: Introduction of a 2-*C*-alkyl and conversion to the 4,5-diol to manipulate ring stereochemistry

To perform a second inversion of a stereocentre the protecting group regiochemistry of *scyllo*-inositol **207**, must be manipulated. It was anticipated that reduction of the orthoester would occur selectively at the 5-*O* (using DIBAL-H,[104,106] see chapter 2.4) to generate the 1,3-*O*-

benzylidene acetal due to the greater directing effect of the equatorial alkyl group than the 2-alkoxyl groups of *myo*-inositol orthoesters. Inversion of the resultant 5-hydroxyl would then regenerate a *myo*-inositol framework, but now with the *C*-alkyl substituent effectively migrated to the 5-*C*. However, it was further hoped that more than one *C*-alkyl derivative could be accessed from one intermediate. Removal of the 6-*O* protecting group R^2 would permit the introduction of a bridging protective group, R^3, such as Markiewicz.[156] Removal of the 4-*O* protecting group R^1 would then generate the 4,5-diol **208** after DIBAL-H reduction. This intermediate allows inversion of both the 4- and 5-hydroxyls. Didehydroxylation of the 4,5-diol to an olefin simultaneously generates two sp^2 centres, **209**.[157] It was expected that from this intermediate, concerted *cis*-dihydroxylation could be carried out on either face of the double bond, by osmylation.[158] Furthermore removal of either of the bridging protecting groups would be expected to direct approach to the opposite face of the inositol ring preferred for steric reasons. Concerted *cis*-dihydroxylation on either face creates one axial and one equatorial hydroxyl restoring the *myo*-geometry. By removal of R^3, it is postulated that concerted *cis*-dihydroxylation from the bottom face introduces diol **212** with the equatorial hydroxyl directly across the ring from the 2-*C*-alkyl, renumbering according to convention (see chapter 1.1), this would give a *myo*-inositol derivative with a 5-*C*-alkyl substituent. Further manipulation of protecting groups is then required before phosphorylation to produce the 5-*C*-alkyl tri- and tetraphosphates. Alternatively if the benzylidene is hydrolysed (see chapter 4.6), osmylation from the top face will introduce a 5-*O*-equatorial hydroxyl to generate diol **213.** Again further manipulation of the protecting groups is required at this stage to generate the protected intermediates for phosphorylation and global deprotection of the required hydroxyls to furnish 4-*C*-alkyl IP_3 and IP_4 analogues.

Unlike the 4-*C*-alkyl intermediates prepared in chapter 3, this synthesis requires different protecting groups on the 4- and 6-*O*, to allow selective introduction of a bridging group on the opposite face to the orthoester. One of these groups is required to be removed selectively in the presence of the other. The introduction of various protecting groups on the 2-, 4- and 6-*O* was reviewed in chapter 2.2, and it is clear that sequential introduction of ether groups by use of sodium hydride or butyl lithium to effect selective axial protections should be possible.

Since, the introduction of a 2-*C*-alkyl substituent and selective reduction of the orthoester to a benzylidene acetal do not require differential protection of the 4- and 6-*O*, a test system was employed to confirm that selective introduction of methylene to the inos-2-ose generated the *exo*-methylene oxide with *scyllo*-geometry. The model system was also used to test selective DIBAL-H reduction of the 2-*O*-alkyl orthobenzoate. It was hoped that selective opening at the 5-*O* would be observed to generate the 1,3-*O*-benzylidene acetal.

6.2 A test system for 2-*C*-alkyl delivery and DIBAL-H reduction

4,6-*O*-Dibenzyl *myo*-inositol orthobenzoate,[159] **214**, was prepared as a test system for alkylation and reduction of the orthobenzoate, Figure 6.4.

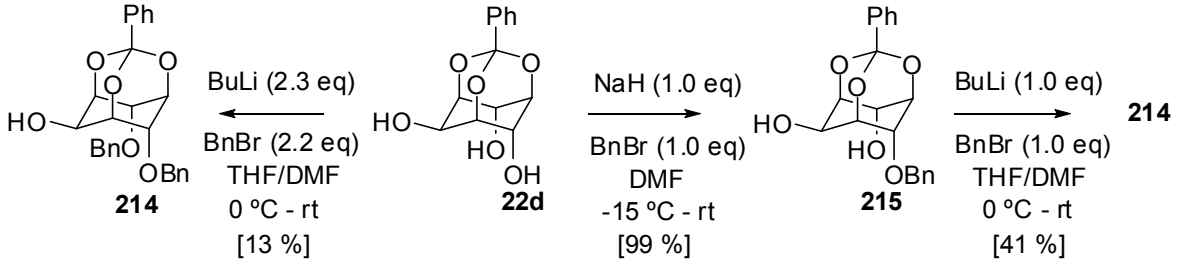


Figure 6.4: Preparation of 4,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate

It was hoped that **214** would be easily prepared in two steps from *myo*-inositol **1**. Unfortunately, despite the reported preference for symmetrical diaxial 4,6-*O*-dibenzylation over 2,6-*O*-dibenzylation with sodium hydride,[159] poor yields of the symmetrical product were obtained for direct benzylation of *myo*-inositol 1,3,5-*O*-orthobenzoate **22d**. Following addition of 2.3 eq. butyl lithium and equilibration at room temperature, desired **214** was obtained in only 13 % yield on addition of benzyl bromide, along with unreacted starting material and unwanted 2,4,6-*O*-tribenzyl ether (13 %) resulting from further deprotonation of the 2-*O*H. As an alternative route, mono-benzyl ether **215** was prepared in almost quantitative yield by deprotonation of the 4-*O*H with 1 eq. sodium hydride followed by addition of benzyl bromide. However, attempts to effect benzylation of the second axial 6-*O*H using butyl lithium and benzyl bromide, only returned desired dibenzyl ether **214** in 41 % yield, alongside a small amount of the 2,4,6-*O*-tribenzyl ether (6 %). The high (89 %) yield reported by Devaraj *et al.*[160] resulting from chelation of lithium in the di-axial 4,6-dioxa-pocket was not replicated, although they also report formation of 5-10 % of tri-*O*-substituted derivatives. These yields were not optimised. Subsequently, 4,6-*O*-dibenzyl orthobenzoate **214** was oxidised using Dess Martin periodinane to generate inos-2-ose **216**, Figure 6.5.

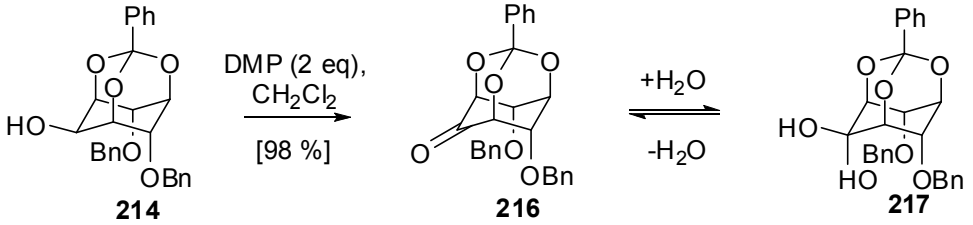


Figure 6.5: Oxidation of the 2-*O*H to the inosose and hydration to the *gem*-diol

As reported[88,91] the inos-2-ose had a tendency to hydrate, forming the *gem*-diol **217**; this had not been observed with the inos-4-ose prepared in chapter 3. The inos-2-ose was dried over

activated molecular sieves and repeatedly evaporated from dry MeCN to dehydrate any *gem*-diol before use in further steps. Upon addition of methyl Grignard to inososе **216** at -78 ºC, selective methyl delivery to the top face of the inososе generated 2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate **218**, Figure 6.6.

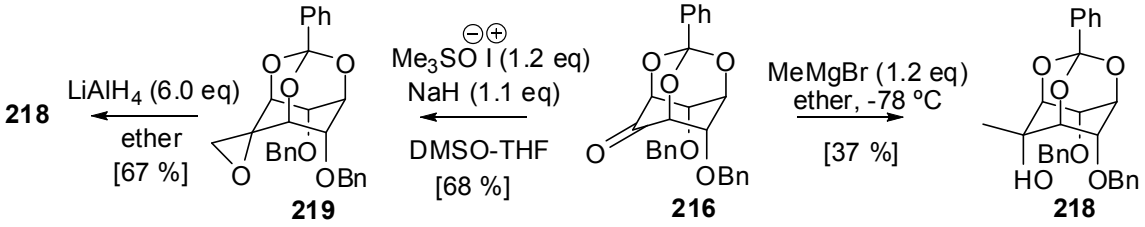


Figure 6.6: Preparation of 2-*C*-methyl by addition of MeMgBr to the inos-2-ose, or by conversion to the *exo*-methylene oxide followed by reduction with $LiAlH_4$

The stereochemistry of methyl addition was confirmed by NOESY in the later DIBAL-H reduction of the orthoester. In order to verify that methylene insertion also occurred by delivery to the top face of the inos-2-ose, *exo*-methylene oxide **219** was prepared. Addition of dimethylsulfoxonium methylide to inososе **216** generated one *exo*-methylene oxide product, **219**, the stereochemistry of which was confirmed by reduction with $LiAlH_4$ to 2-*C*-methyl orthobenzoate **218**, Figure 6.6.

Upon treatment of **218** with 3 eq. DIBAL-H two products were observed, although the reaction did not go to completion. Isolation of the two products by chromatography demonstrated that only one benzylidene acetal was present, alongside the 2,3,5-triol **221** resulting from a second hydride delivery to the orthobenzoate. Triol **221** had a clear cross-peak between the 2-CH_3 and both the 4- and 6-*H* in the NOESY spectrum, confirming that *scyllo*-geometry had resulted from methyl addition. The ratio of compounds as judged by ^{1}H-NMR of the crude was 3:2:1 (starting material-acetal-triol), Figure 6.7.

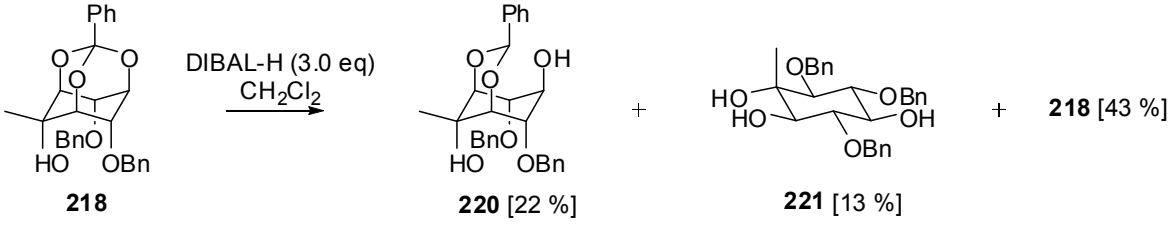


Figure 6.7: Reduction of 2-*C*-methyl orthobenzoate with limited DIBAL-H

Benzylidene acetal **220** was symmetrical, confirming that the expected regioselective hydride insertion had occurred to the 5-*O*-C bond. This result suggests that the benzylidene acetal generated is not significantly more stable than the orthobenzoate towards DIBAL-H reduction. It has been postulated in chapter 4 that the steric effect of the methyl group may not be very large (compared to e.g. a benzyl ether or larger *C*-alkyl group). Therefore it is hoped that with larger 2-

C-alkyl substituents, the tendency for over-reduction would be reduced, as was observed for the larger 4-*C*-alkyl compounds in chapter 4.5.

6.3 Orthogonal protection of the 4- and 6-*O*

The test system demonstrated that 2-*C*-alkyl introduction via an *exo*-methylene oxide selectively generates a *scyllo*-inositol intermediate and that DIBAL-H reduction could prepare (in moderate yield) the desired 1,3-*O*-benzylidene acetal. The subsequent manipulations required protection of the 4- and 6-*O*H with two different groups. Therefore 4-*O*-allyl-6-*O*-*p*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **222** was prepared in three steps, from *myo*-inositol, Figure 6.8.

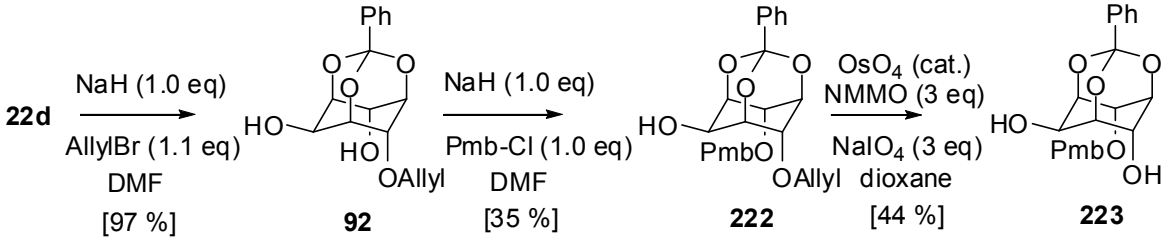


Figure 6.8: Preparation of orthogonally protected 4-*O*-allyl-6-*O*-Pmb-*myo*-inositol 1,3,5-*O*-orthoformate.

4-*O*-Allyl **92** was prepared as described in section 2.6. Subsequent addition of sodium hydride followed by *p*-methoxybenzyl chloride generated **222** in low (35 %) yield, unoptimised. 2,6-*O*-Di(*p*-methoxybenzyl)-4-*O*-allyl-*myo*-inositol 1,3,5-*O*-orthoformate **224** (10 %) and unreacted **92** (11 %) were also isolated upon purification. None of the possible 2-*O*-(*p*-methoxybenzyl) side product was observed, suggesting that deprotonation with sodium hydride occurred preferentially at the 6-*O*H. The selective removal of the 4-*O*-allyl protecting group was demonstrated by addition of 4-methyl morpholine *N*-oxide and OsO_4 followed by $NaIO_4$ to generate **223**.[161] Oxidation of **222** with Dess Martin periodinane quantatively generated the inos-2-ose **225**, Figure 6.9.

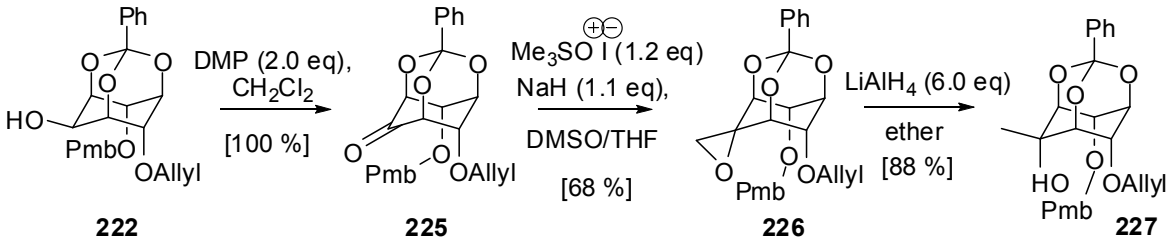


Figure 6.9: Oxidation of **222** to the inos-2-ose followed by stereoselective introduction of a 2-*C*-methyl substituent via *exo*-methylene oxide **226**.

Inos-2-ose **225** was selectively converted to the *scyllo*-inositol *exo*-methylene oxide, **226**, by addition of dimethylsulfoxonium methylide. Subsequent reduction with 2 eq. $LiAlH_4$ afforded

2-*C*-methyl *scyllo*-inositol **227**. Prolonged treatment of **227** with 6 eq. $LiAlH_4$ led to the formation of a lower R_f side product, isolated and identified as **228**, the product of reductive cleavage of the 4-*O*-allyl ether (51 %). Alternatively, the 4-*O*-allyl ether could be selectively removed in the presence of the 6-*O*-Pmb ether, by oxidation of the double bond to the diol using OsO_4, followed by treatment with $NaIO_4$.[161] The majority of the resultant formate ester was cleaved during work up to generate **228**, and the remaining portion was treated with triethylamine in ethanol to remove the formate ester, Figure 6.10.

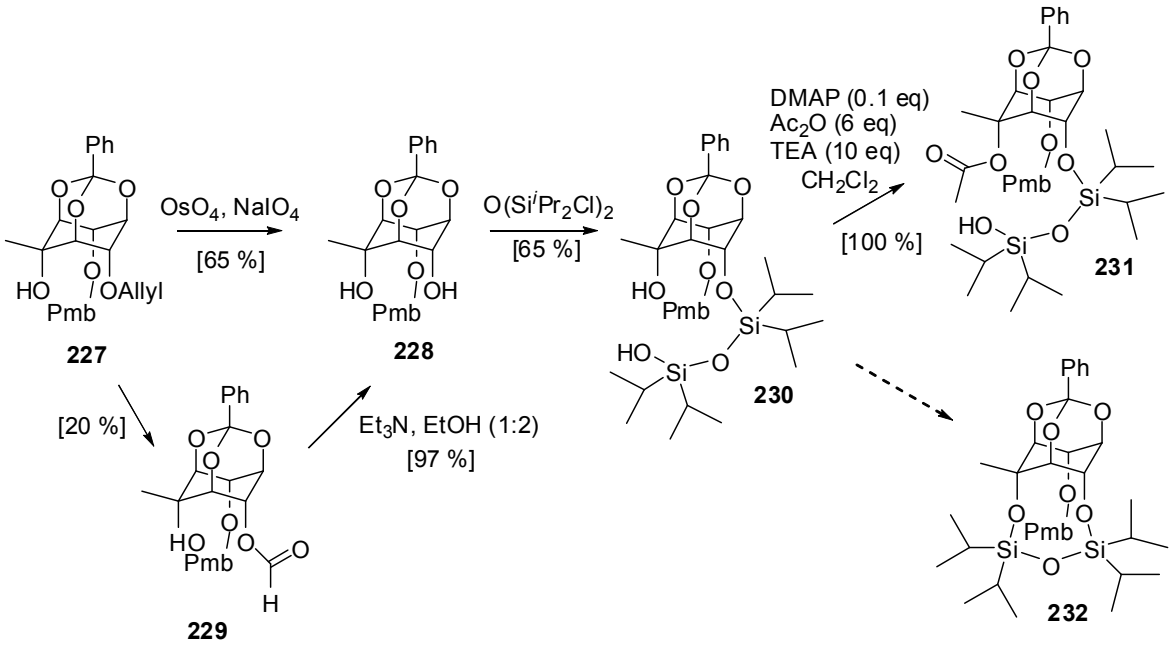


Figure 6.10: Removal of the 4-*O*-allyl followed by attempted introduction of a 1,3-diaxial bridging group

The apparent reductive removal of the allyl group with $LiAlH_4$ was unexpected, and it is not understood why this group was selectively cleaved in the presence of the Pmb group. This demonstrated that the 4-*O*-allyl ether could be removed either simultaneously with reduction of the *exo*-methylene oxide, or as a separate step. For larger 2-*C*-alkyl groups, where $LiAlH_4$ reduction is not required, the 4-*O*-allyl could be removed by either set of conditions, providing synthetic flexibility.

A bridging protective group was then required for 2,4-diol **228** for which Markiewicz reagent (1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane)[156] was selected as it is known to react with 1,3-diols to give an 8-membered ring. Upon addition of Markiewicz to 2,4-diol **228**, a single product was observed, **230**. It was confirmed by MS and subsequent acylation of the free 2-*O*H that the cyclisation of the Markiewicz reagent to the tertiary 2-*O*H to prepare the desired **230** had not occurred. This reaction has not yet been thoroughly explored and it may be possible to force it to completion or to employ an alternative bridging protecting group.

6.4 Concluding Remarks

The lessons learnt from the 4-*C*-alkyl system have been successfully applied to this more elaborate synthetic route. The test system, and subsequent reactions on the orthogonally protected inos-2-ose **223** have demonstrated the introduction of a 2-*C*-methyl in the preparation of **225** both directly from the inososе and via key *exo*-methylene oxide **224**. It is anticipated that this *exo*-methylene oxide can be opened with larger alkyl substituents in the same way as amply demonstrated for 4-*C*-*exo*-methylene oxide **98** in chapter 3, to create a range of 2-*C*-alkyl inositides. It has also been demonstrated that the 4-*O*-allyl ether can be selectively cleaved in the presence of the 6-*O*-Pmb ether, orthoester and 2-*C*-methyl substituent to generate a diol with free 2- and 4-*O*H. Initial unsuccessful attempts to introduce the bridging Markiewicz protecting group need further investigation. However, following the experiments on the test system it is expected that the resulting *scyllo*-inositol intermediate **230** will undergo regiocontrolled DIBAL-H insertion at the 5-*O*-C bond. The elimination and reintroduction of the 4,5-diol still need to be probed to confirm that they occur selectively as predicted. The subsequent steps after reintroduction of *myo*-stereochemistry involve protecting group manipulations, phosphorylation and deprotection. It is expected that these steps will proceed in a similar manner to the 4-*C*-alkyl system in the preceding chapters to prepare the final *C*-alkyl inositol phosphate analogues.

Conclusions

The aim of this thesis was to prepare 4-*C*-alkyl inositol phosphates. This has been successfully achieved and a small library of both 4-*C*-alkyl Ins(1,4,5)P_3 and 4-*C*-alkyl Ins(1,3,4,5)P_4 analogues have been prepared. The preparation of *C*-alkyl analogues for use in RLE is therefore certainly feasable. At each stage of the synthesis, the chemical limitations have been explored, and these were evaluated in the concluding remarks at the end of each chapter.

A wide range of 4-*C*-alkyl groups were introduced by nucleophilic opening of the *exo*-methylene oxide using alkyl lithium cuprates in chapter three to generate a series of 4-*C*-alkyl-2,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoates. It has been proven that it is possible to introduce large alkyl and aryl substituents including 4-*C*-benzyl and -(2-naphthyl)methyl groups. The 1,3,5-*O*-orthobenzoate was manipulated in chapter four using either acidic hydrolysis or reduction with DIBAL-H to selectively reveal the hydroxyls required for phosphorylation, generating 4-*C*-alkyl triols and tetrols. Tri- or tetraphosphorylation was then carried out in chapter five using P(III) phosphoramidites with subsequent oxidation using *m*CPBA. Global deprotection of benzyl groups then furnished the final target compounds. Time did not permit the phosphorylation of all 4-*C*-alkyl derivatives. However, the successful phosphorylation of 4-*C*-benzyl IP_3 and IP_4 demonstrated that phosphorylation of hindered tertiary centres is possible with a less sterically demanding P(III) phosphoramidite. The limitation of the methods developed here may be phosphorylation of β-tertiary centres such as the 4-*C*-neopentyl substituent. However, such bulky groups may be more amenable to phosphorylation if a larger alkyl spacer was placed between the bulky substituent and the inositol ring.

The initial investigations to introduce a *C*-alkyl substituent on a wider variety of positions in chapter six, demonstrated that preparation of an *exo*-methylene oxide and subsequent nucleophilic opening can be applied to other positions on the inositol ring. Although further work is required to reintroduce the *myo*-geometry, the generation of multiple *C*-alkyl derivatives from a common intermediate appeared promising.

A new class of IP_n head group has been prepared in this thesis. These analogues have a wide range of potential applications. An *exo*-methylene oxide at any position on the ring could be opened with a wide range of nucleophiles, to introduce a vast array of functionalities. Cross-linkers, fluorescent markers and probes could be introduced on the carbon backbone using this method, rather than as has been reported on the inositol hydroxyls.[56] Addition of these species to the carbon framework will not impact the coulombic and H-bonding interactions as the phosphate and hydroxyl groups are left in tact. If the 3-, 4- and 5-*C* are most suitable for addition of steric

protrusions, the 2- and 6-*C* may be suited to introduction of additional functionalities such as fluorescent markers without impinging on binding affinity.

The smaller 4-*C*-alkyl IP_3 and IP_4 derivatives which have been prepared in this thesis, are not expected to be suitable for RLE applications, as the alkyl groups would be insufficiently large to prevent binding to the WT protein. However, there are alternative uses for these compounds. As research into phosphoinositide biology expands, small molecules which differ only slightly from the natural ligands are potential selective agonists or antagonists for their WT protein binding sites. The reduced Ca^{2+} release observed from the interaction of IP_3Rs with 4-*C*-methyl IP_3, suggests that alternative small 4-*C*-alkyl IP_3s may act as antagonists which could bind to the IP_3Rs and prevent Ca^{2+} release. The experimentally determined selective inhibition of the 5-phosphatase domain of synaptojanin with 4-*C*-ethyl IP_4, also suggests that the smaller 4-*C*-alkyl analogues may be useful probles for elucidating the mechanism of action of this double phosphatase. These are only the results from two initial experiments and there may be many more applications in the future for this series of compounds.

Outlook

Following the work presented in this thesis, it cannot be denied that the preparation of a *C*-substituted IP_n is certainly possible. There are still areas which require further investigation, to ensure the successful preparation of any *C*-alkyl substituent.

Continued study of the general approach in chapter six towards the preparation of 4-, 5- and 6-*C* alkyl substituents would generate a wide variety of *C*-alkyl-IP_ns. This would provide three new sites for the introduction of substituents for biological studies and prove the generalisability of *C*-alkyl introduction, phosphorylation and deprotection.

Time did not permit a full investigation of the reaction of less sterically demanding cyclic phosphoramidite, *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane with larger *C*-alkyl triols or tetrols. There are alternative reagents which could be employed to effect this transformation, such as the phosphorochloridites. Di(2-cyanoethyl)phosphorochloridite did successfully phosphorylate 4-*C*-benzyl tetrol and this may be a more reactive reagent for phosphitylating hindered tertiary hydroxyls. However, the conditions for the two step deprotection of the phosphate cyanoethyl and hydroxyl benzyl ether groups would need to be resolved. The choice of hydrogenation catalyst, and perhaps the use of a homogenious catalyst may improve the deprotection of the final 2- and 6-*O*-benzyl ethers.

The range of *C*-substituents is not limited to alkyl and aryl groups. Other nucleophiles could be used to open the *exo*-methylene oxide to generate for example *O*- or *N*-linked alkyl groups. Furthermore, *C*-alkyl or aryl substituents could be manipulated after they are introduced, for example the proposed route to 4-*C*-alkyl IP_4 analogues suitable for ion-pair exchange RLE from 4-*C*-[3-phenyl(prop-2-enyl)] at the end of chapter three. A considerable amount of time could have been spent in the introduction of such groups. However, this was beyond the scope this project. Now that the chemistry is in place to reach the final targets, such alternative 'bumps' could be explored.

The ultimate targets for RLE protein-ligand binding pairs are the full PIP_n lipids, and this will require a synthetic strategy which differentiates between the 1-*O* and remaining positions requiring phosphorylation, in order to introduce the phosphatidate group. They will also require a resolution step at some stage in the synthesis, for example by preparation and separation of the camphanate esters[73] of the 2,6-*O*-dibenzyl orthoester as the species prepared in this thesis are racemic. Both these challenges have been overcome in the reported chiral syntheses of PIP_n[145] and therefore should be readily applied to the 4-*C*-alkyl analogue synthesis reported here.

Chapter 7: Experimental

The experimental details for the compounds prepared in this thesis are divided by chapter (three to six), and ordered sequentially by number within each chapter. Side products are included alongside the main reaction from which they were generated. A number of preparation methods were developed and used to effect a series of transformations. To prevent repetition, these have been included in section 7.2, entitled General Methods, before the main experimental sections 7.3-7.6.

7.1 General Experimental and spectroscopic methods

All reactions were conducted at room temperature in dry glassware under an atmosphere of nitrogen unless otherwise stated. All chemicals were used directly from the supplier's vessel unless otherwise stated. Reagents were purchased from *Sigma-Aldrich Ltd* or *Acros Organics*. All evaporation of organic solvents was carried out using a water-cooled Buchi rotary evaporator. Solvents were distilled before use, after drying over a suitable reagent, and stored under nitrogen in sealed containers over 4 Å molecular sieves.

^{1}H-NMR spectra were recorded on Bruker AC-270, AV-400 or AV-500 spectrometers. Chemical shifts (δ) are quoted in parts per million (ppm) relative to an internal solvent standard. The splitting patterns for ^{1}H-NMR spectra are denoted as follows; s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad) and combinations thereof. Coupling constants (*J*) are quoted in Hertz (Hz). ^{13}C-NMR spectra were recorded at 100 or 125MHz. Chemical shifts are again given in parts per million. Peaks are assigned as C (quaternary), CH (tertiary), CH_2 (secondary) and CH_3 (primary). Assignments were made with the aid of DEPT90 and 135, HSQC, COSY and NOESY experiments. ^{13}C and ^{31}P-NMR are proton decoupled. Mass spectra were recorded on a *VG* AutoSpec-Q (CI) or a *Micromass* LCT Premier (ESI) spectrometer. Infra-red spectra were recorded on KBr disks as solutions in CH_2Cl_2. Analytical TLC was carried out on *Merck* silica gel 60 F_{254} glass-backed plates. Plates were visualised using UV light or developed using potassium permanganate ($KMnO_4$). Flash chromatography was carried out using flash silica from *Merck*.

7.2 General Methods

General method 1: Synthesis of 2,6-*O*-dibenzyl-4-C-alkyl-*myo*-inositol 1,3,5-*O*-orthobenzoates using alkyl lithium cuprates.

CuCN (4.0 mmol) was evaporated from toluene (3 × 2 mL), taken up in ether (5 mL) and cooled to -78 ºC. Alkyl lithium (8.0 mmol) was added dropwise to the stirred suspension which turned pale yellow. In a separate vessel, 2,6-*O*-dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-

O-orthobenzoate (1.0 mmol) was azeotroped with toluene (3 × 2 mL), taken up in ether (5 mL) and cooled to -78 ºC. The methylidene solution was added slowly to the alkyl lithium cuprate solution and stirred for 4 h, at -78 ºC, or until complete as judged by TLC visualised with $KMnO_4$ stain. Excess reagent was quenched with NH_4Cl-NH_4 (9:1 v/v, 10 mL) and the two-phase solution stirred for a further 30 min before addition of brine (5 mL). The aqueous phase was washed with ether (3 × 50 mL) and the combined organic layers dried ($MgSO_4$) and evaporated to dryness under reduced pressure.

General method 2: Total reduction of *myo*-inositol orthobenzoates with DIBAL-H.

The *myo*-inositol 1,3,5-*O*-orthobenzoate (0.50 mmol) was evaporated from toluene (3 × 2 mL), taken up in CH_2Cl_2 (5 mL) and cooled to -78 °C. DIBAL-H (0.7 M in hexanes, 1.75 mmol) was added drop-wise, the solution warmed to rt over 3 h and stirred for 24 h. The reaction was quenched by drop-wise addition of H_2O (25 mL) and extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were washed with H_2O (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and evaporated to dryness under reduced pressure.

General method 3: Reduction of the 4-*C*-alkyl orthobenzoates using excess DIBAL-H.

2,6-*O*-Dibenzyl-4-*C*-alkyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (0.21 mmol) was evaporated from MeCN (3 × 1 mL), taken up in CH_2Cl_2 (1 mL) and cooled to -78 ºC. DIBAL-H (1.0 M solution in hexanes, 0.84 mmol) was added drop-wise at -78 ºC. The solution was allowed to warm to rt over 2 h. The reaction was monitored by TLC (EtOAc-hexane, 3:7 v/v) and quenched by drop-wise addition of H_2O (5 mL) when all starting material was consumed. The product was taken up in CH_2Cl_2 (10 mL), and separated from the aqueous layer, which was subsequently washed with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with sat. $NaHCO_3$, and then brine. The organic layer was dried ($MgSO_4$) and all solvents evaporated under reduced pressure.

General method 4: Acidic hydrolysis of orthobenzoate to mixture of benzoyls

2,6-*O*-Dibenzyl-4-*C*-alkyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (0.2 mmol) was taken up in conc. HCl-(propan-1-ol) (1:2 v/v, 3 mL) and refluxed for 3 h. The solution was cooled, neutralised with sat. $NaHCO_3$ and the products extracted into EtOAc (× 2). The combined organic layers were washed with H_2O then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure.

General method 5: Removal of benzoyls under basic conditions to yield the 4-*C*-alkyl tetrol

The crude mixture of benzoyls (0.2 mmol) was evaporated from MeCN (3 × 1 mL) and taken up in MeOH (2 mL). After addition of NaOMe (25% solution in MeOH, 0.15 mL, 0.1 mmol) the solution was heated to reflux for 3 h. The resulting solution was cooled, neutralised by

drop-wise addition of 4 M HCl and all solvents removed under reduced pressure. The crude material was taken up in dry MeOH and the salts removed by filtration under gravity. The mother liquor was evaporated to dryness under reduced pressure.

General method 6: Phosphorylation of triols using *N,N*-diisopropyldibenzyl phosphoramidite

2,3,6-*O*-Tribenzyl-4-*C*-alkyl-*myo*-inositol (0.1 mmol) and 1-*H*-tetrazole (1.2 mmol) were evaporated from MeCN (3 × 2 mL), taken up in MeCN (5 mL) and *N,N*-diisopropyldibenzyl phosphoramidite (0.6 mmol) added. After 2 h the solution was cooled to -40 ºC and *m*CPBA (0.7 mmol) added. Stirring was continued at 0 ºC for 2 h. The solution was diluted with CH_2Cl_2, washed with 10 % aq. $Na_2S_2O_3$, sat. $NaHCO_3$, H_2O and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was stirred with 1 M I_2 in pyridine-H_2O-THF (2:1:7 v/v, 3 mL) for 15 mins before dilution with H_2O and $CHCl_3$. $Na_2S_2O_3$ was added to the vigourously stirred solution until both layers were clear in colour. The organic layer was washed with sat. $NaHCO_3$, then HCl (0.4 M), dried ($MgSO_4$) and all solvents evaporated under reduced pressure.

General method 7: Phosphorylation of tetrols using *N,N*-diisopropyldibenzyl phosphoramidite

2,6-*O*-Dibenzyl-4-*C*-alkyl-*myo*-inositol (0.1 mmol) and 1-*H*-tetrazole (1.4 mmol) were evaporated from MeCN (3 × 2 mL), taken up in MeCN (5 mL) and *N,N*-diisopropyldibenzyl phosphoramidite (0.7 mmol) added. After 2 h the solution was cooled to -40 ºC and *m*CPBA (1.4 mmol) added. Stirring was continued at 0 ºC for 2 h. The solution was diluted with CH_2Cl_2, washed with 10 % aq. $Na_2S_2O_3$, sat. $NaHCO_3$, H_2O and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was stirred with 1 M I_2 in pyridine-H_2O-THF (2:1:7 v/v, 3 mL) for 15 mins before dilution with H_2O and $CHCl_3$. $Na_2S_2O_3$ was added to the vigourously stirred solution until both layers were clear in colour. The organic layer was washed with sat. $NaHCO_3$, then HCl (0.4 M), dried ($MgSO_4$) and all solvents evaporated under reduced pressure.

General method 8: Phosphorylation of tetrols using *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane

2,6-*O*-Dibenzyl-4-*C*-alkyl-*myo*-inositol (0.1 mmol) and 1-*H*-tetrazole (1.4 mmol) were evaporated from MeCN (3 × 2 mL), taken up in MeCN (1 mL) and placed in a water bath at 25 ºC. *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane[121] (0.9 mmol) was added in one batch. After 2 h the solution was cooled to 0 ºC and *m*CPBA (1.4 mmol) added. Stirring was continued at 0 ºC for 2 h. The solution was diluted with CH_2Cl_2, washed with 10 % aq. $Na_2S_2O_3$, sat. $NaHCO_3$, H_2O

and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure.

General method 9: Global deprotection of benzyl groups using H_2 and Pd(black): IP_3 precursors

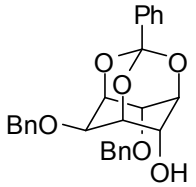
1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-alkyl-*myo*-inositol (0.05 mmol) was taken up in ^{t}BuOH-H_2O (6:1 v/v, 6 mL) to which was added $NaHCO_3$ (0.41 mmol) and Pd-black (1.03 mmol). The solution was stirred under an atmosphere of H_2 for 36 h. The catalyst was filtered off, washed with H_2O (4 × 10 mL) and the mother liquor concentrated under reduced pressure, before being taken up in H_2O, washed with CH_2Cl_2 (× 2) and freeze dried.

General method 10: Global deprotection of benzyl groups using H_2 and Pd(black): IP_4 precursors

1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-alkyl-*myo*-inositol (0.25 mmol) was taken up in ^{t}BuOH-H_2O (6:1 v/v, 54 mL) to which was added $NaHCO_3$ (1.98 mmol) and Pd-black (4.95 mmol). The solution was stirred under an atmosphere of H_2 for 36 h. The catalyst was filtered off, washed with H_2O (4 × 10 mL) and the filtrate concentrated under reduced pressure. The remaining solution was taken up in H_2O, washed with CH_2Cl_2 (× 2) and freeze dried. The powdery solid was redissolved in the minimum volume of H_2O and passed through DOWEX 50WX8-200 H^+ resin. Acidic fractions of eluent were combined, neutralised with aq. NH_3 and freeze dried.

7.3 Chapter 3

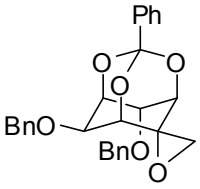
2,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (95)



2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-*O*-orthobenzoate (**96**, 100 mg, 0.22 mmol) was evaporated from MeCN (1 mL × 3) and taken up in diethyl ether (1 mL). The solution was cooled to -78°C, ethylmagnesium bromide (1.0 M solution in THF, 0.27 mL, 0.27 mmol) was added drop-wise and the reaction stirred for 30 min before warming to rt. After 2 h the reaction was quenched by drop-wise addition of H_2O (2 mL) and the product extracted into CH_2Cl_2. The organic layer was washed with HCl (0.1 M ×2), sat. $NaHCO_3$, H_2O and brine, then dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The *title compound* (**95**, 100 mg, 99 %) was obtained as a pale yellow oil; R_f (EtOAc-hexane, 1:4 v/v) 0.16; δ_H (400 MHz, $CDCl_3$) 7.66-7.19 (15H, m, 15 × Ar ***H***),

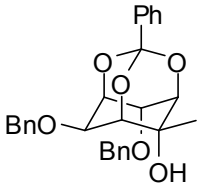
4.80 (1H, d, *J* 12.5), 4.68 (1H, d, *J* 12.4) (2 × OCH***H***Ph), 4.58-4.53 [4H, m, (2 × Ins ***H***) + OC***H₂***Ph], 4.50-4.47 (2H, m), 4.40-4.38 (1H, m), 3.94-3.93 (1H, m) (4 × Ins ***H***), 3.63 (1H, bs, Ins 4-O***H***) ppm; δ_C (125 MHz, $CDCl_3$) 137.81, 136.79, 135.93 (3 × Ar ***C***), 129.46, 128.84 (2C), 128.75, 128.53 (2C), 128.11 (2C), 127.93 (5C), 125.37 (2C) (15 × Ar ***C***H), 107.32 (Ph***C***O_3), 74.46, 73.41 (2 × Ins ***C***H), 72.96 (O***C***H_2Ph), 71.29 (Ins ***C***H), 70.99 (O***C***H_2Ph), 68.67, 67.94, 65.07 (3 × Ins ***C***H) ppm; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 447.1793 (100), $C_{27}H_{27}O_6$ requires 447.1808.

2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (98)



To a clear solution of trimethylsulfoxonium iodide (109 mg, 0.49 mmol) in DMSO (1 mL) was added NaH (60 % dispersion in mineral oil, 21 mg, 0.54 mmol) and the mixture stirred for 40 min. 2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-*O*-orthobenzoate (**96**, 200 mg, 0.45 mmol) was evaporated from MeCN (1 mL × 3), taken up in THF (1 mL) and added to the reaction mixture, which was stirred at rt for 1 h. The reaction was quenched by drop-wise addition of H_2O (2 mL) and the product taken up in CH_2Cl_2. The organic layer was washed with sat. $NaHCO_3$, H_2O, then brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 2:5 v/v) afforded the *title compound* (**98**, 184 mg, 89 %) as a clear oil; R_f (EtOAc- hexane, 1:1 v/v) 0.70; δ_H (400 MHz, $CDCl_3$) 7.68-7.26 (15H, m, 15 × Ar ***H***), 4.75 (1H, d, *J* 12.2), 4.70 (1H, d, *J* 12.1), 4.68 (1H, d, *J* 11.8) (3 × OCH***H***Ph), 4.55 (1H, dq, *J* 4.2, 2.0, Ins 1-***H***), 4.53 (1H, d, *J* 11.9, OCH***H***Ph), 4.42 (1H, t, *J* 3.8 Ins 6-***H***), 4.03 (1H, t, *J* 1.7 Ins 2-***H***), 3.95-3.94 (2H, m, Ins 3-***H*** + Ins 5-***H***), 2.95 (1H, d, *J* 4.7), 2.93 (1H, d, *J* 4.7) (2 × Ins 4-CCH***H***) ppm; δ_C (100 MHz, $CDCl_3$) 137.76, 137.25, 136.74 (3 × Ar ***C***), 129.55, 128.56 (2C), 128.46 (2C), 128.10, 127.99 (2C), 127.84 (3C), 127.79 (2C), 125.41 (2C) (15 × Ar ***C***H), 108.14 (Ph***C***O_3), 74.85, 72.50, 72.34, 71.55 (4 × Ins ***C***H), 71.46, 71.25 (2 × O***C***H_2Ph), 68.29 (Ins ***C***H), 54.92 (Ins ***C***), 51.01 (Ins 4-C***C***HH) ppm; MS (ESI^+) *m/z* (%) found $[M+H]^+$ 459 (62), $[M+Na]^+$ 481 (100); HRMS (ESI^+) found $[M+H]^+$ 459.1800, $C_{28}H_{27}O_6$ requires 459.1808.

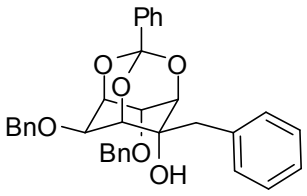
2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol-1,3,5-*O*-orthobenzoate (115)[130]



By addition of MeMgBr to the inos-4-ose. 2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-*O*-orthobenzoate (**96**, 100 mg, 0.225 mmol) was evaporated from MeCN (1 mL × 3), taken up in diethyl ether (1 mL) and the solution cooled to -78 °C. Methylmagnesium bromide (3.0 M solution in diethyl ether, 90 μL, 0.27 mmol) was added drop-wise and the solution stirred for 20 min before warming to rt. After 2 h the reaction was quenched by drop-wise addition of H_2O (2 mL). The crude product was extracted into CH_2Cl_2, washed with 0.1 M HCl, sat. $NaHCO_3$ and brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with ether-hexane (0:1 → 3:10 v/v) afforded the *title compound* (**115**, 66 mg, 64 %) as a clear oil.

By reduction of the *exo*-methylene oxide. 2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 150 mg, 0.33 mmol) was evaporated from MeCN (1 mL × 3), taken up in ether (2 mL) and added drop-wise to a suspension of $LiAlH_4$ (86 mg, 2.26 mmol) in ether (1 mL). After 45 min the reaction was quenched by drop-wise addition of EtOAc. The resulting solution was diluted with ether and washed with HCl (0.5 M, 10 mL, × 2), sat. $NaHCO_3$, H_2O and then brine. The organic layer was dried ($MgSO_4$) and all solvents evaporated under reduced pressure to afford the *title compound* (**115**, 70 mg, 46 %) as a clear oil: R_f (hexane-ether, 3:7 v/v) 0.70; δ_H (400 MHz, $CDCl_3$) 7.65-7.20 (15H, m, 15 × Ar ***H***), 4.80 (1H, d, *J* 12.5), 4.66 (1H, d, *J* 12.5), 4.60 (1H, d, *J* 11.6), 4.55 (1H, d, *J* 11.6) (4 × OCH***H***Ph), 4.50 (1H, t, *J* 3.9, Ins 6-***H***), 4.44 (1H, dq, *J* 3.6, 1.7, Ins 1-***H***), 4.29 (1H, s, Ins 4-O***H***), 4.17 (1H, q, *J* 3.7, 1.8, Ins 3-***H***), 4.02 (1H, dt, *J* 3.8, 1.9, Ins 5-***H***), 3.99 (1H, t, *J* 1.8, Ins 2-***H***), 1.64 (3H, s, Ins 4-C***H₃***) ppm; δ_C (100 MHz, $CDCl_3$) 137.89, 136.94, 135.87 (3 × Ar ***C***), 129.44, 128.84 (2C), 128.75, 128.51 (2C), 128.10 (2C), 127.98 (2C), 127.93 (3C), 127.88, 125.35 (15 × Ar ***C***H), 107.31 (Ph***C***O_3), 77.29, 74.70 (2 × Ins ***C***H), 72.96 (O***C***H_2Ph), 72.89 (Ins ***C***H), 71.04 (O***C***H_2Ph), 70.66 (Ins ***C***H), 69.58 (Ins ***C***), 66.22 (Ins ***C***H), 24.27 (Ins 4-***C***H_3) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 461 (100), $[M+Na]^+$ 483 (93); HRMS (ESI^+) found 461.1959 (100), $C_{28}H_{29}O_6$ requires 461.1964.

2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (125)



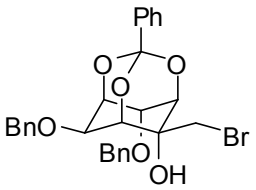
By addition of benzyl magnesium chloride to the inososе. 2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-*O*-orthobenzoate (**96**, 105 mg, 0.24 mmol) was evaporated from toluene (3 × 1 mL), taken up in ether (1 mL) and cooled to -78 ºC. In a separate vessel, to magnesium turnings (32 mg, 1.33 mmol) in ether (1 mL) was added benzyl chloride (153 μL, 1.33 mmol). The cloudy suspension was cooled to -78 ºC and added to the inososе solution. After 30 mins the solution was warmed to

rt for a further 2 h. The reaction was quenched with H_2O, diluted with ether and washed with H_2O, then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 2:3 v/v) afforded two products, the *title compound* (**125**, 13 mg, 10 %) and 2,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**95**, 30 mg, 28 %) both as pale yellow oils.

By addition of phenyl lithium to the *exo*-methylene oxide. 2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 300 mg, 0.65 mmol) was evaporated from toluene (3 × 3 mL) and taken up in ether (3 mL). Phenyl lithium (1.8 M in dibutyl ether, 724 µL, 1.30 mmol) was added drop-wise to the stirred solution. After 6 h the reaction was quenched with H_2O (1 mL), diluted with ether and washed with H_2O (× 2), then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material (732 mg) was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 3:7 v/v) afforded the *title compound* (**125**, 231 mg, 66 %) as a pale yellow oil.

By addition of phenyl lithium cuprate to the *exo*-methylene oxide. 2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 970 mg, 2.10 mmol) was treated with phenyl lithium cuprate according to general method 1. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 3:7 v/v) afforded the *title compound* (**125**, 1.02 g, 90 %) as a clear oil; R_f (EtOAc-hexane, 3:7 v/v) 0.63; δ_H (400 MHz, $CDCl_3$) 7.78-7.75 (2H, m), 7.45-7.29 (16H, m), 7.17-7.14 (2H, m) (20 × Ar ***H***), 4.78 (1H, d, *J* 12.2), 4.64 (1H, d, *J* 12.2), 4.54 (1H, d, *J* 11.7) (3 × OCH***H***Ph), 4.51 (1H, t, *J* 3.9, Ins 6-***H***), 4.49 (1H, d, *J* 11.2, OCH***H***Ph), 4.48 (1H, dq, *J* 3.9, 2.0, Ins 1-***H***), 4.24 (1H, q, *J* 2.0, Ins 3-***H***), 4.23 (1H, s, Ins 4-O***H***), 4.01 (1H, t, *J* 1.5, Ins 2-***H***), 3.97 (1H, dt, *J* 3.4, 1.5, Ins 5-***H***), 3.42 (1H, d, *J* 13.7), 3.27 (1H, d, *J* 13.7) (2 × CH***H***Ph) ppm; δ_C (125 MHz, $CDCl_3$) 137.83, 137.02, 135.92, 135.76 (4 × Ar ***C***), 131.04 (2C), 129. 49, 128.76 (2C), 128.69, 128.46 (2C), 128.11 (2C), 128.04 (2C), 128.02 (2C), 127.95 (2C), 127.86, 126.51, 125.41 (2C) (20 × Ar ***C***H), 107.44 (Ph***C***O_3), 75.50, 74.58 (2 × Ins ***C***H), 73.03, 71.11 (2 × O***C***H_2Ph), 71.09 (Ins ***C***), 70.81, 70.48, 66.36 (3 × Ins ***C***H), 41.62 (***C***H_2Ph) ppm; HRMS (CI^+) *m/z* (%) found $[M+H]^+$ 537.2296 (100), $C_{34}H_{33}O_6$ requires 537.2277.

2,6-*O*-Dibenzyl-4-*C*-bromomethyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (126)

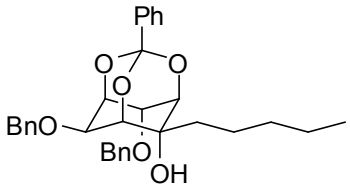


By treatment of *exo*-methylene oxide with MeMgBr: 2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 100 mg, 0.22 mmol) was evaporated from MeCN (1 mL × 3) and taken up in THF (1 mL). The solution was cooled to -78 °C, MeMgBr (3.0

M solution in diethyl ether, 80 μL, 0.24 mmol) was added drop-wise and the reaction stirred for 30 min before being warmed to rt. The reaction was quenched by drop-wise addition of H_2O (2 mL) and taken up in CH_2Cl_2. The organic layer was filtered through celite, washed with H_2O and then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure to afford the *title compound* (**126**, 62 mg, 53 %) as a colourless oil.

By treatment of *exo*-methylene oxide with β-styrene lithium cuprate; 2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 100 mg, 0.22 mmol) was treated with β-styrene lithium cuprate according to the general method 1, using β-lithium styrene prepared according to the following method; β-bromo-styrene was taken up in ether-toluene (1:1 v/v, 2 mL) and cooled to -20 ºC. *n*-Butyl lithium (1.0 M solution in hexanes, 1.95 mL, 1.95 mmol) was added dropwise and the solution maintained at -20 ºC for a further 30 mins, before being cooled to -78 ºC for use in general method 1. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:0 → 3:7 v/v) afforded the *title compound* (**126**, 60 mg, 51 %) as a colourless oil; R_f (EtOAc-hexane, 3:7 v/v) 0.56; δ_H (400 MHz, $CDCl_3$) 7.66-7.23 (15H, m, 15 × Ar ***H***), 4.82 (1H, d, *J* 12.0), 4.70 (1H, d, *J* 12.4), 4.64 (1H, d, *J* 11.6), 4.58 (1H, d, *J* 11.6) (4 × OCH***H***Ph), 4.55 (1H, t, *J* 4.0, Ins 6-***H***), 4.47 (1H, s, Ins 4-O***H***), 4.45 (2H, m, Ins 1-***H*** + Ins 3-***H***), 4.38 (1H, dt, *J* 3.6, 2.0, Ins 5-***H***), 4.08 (1H, d, *J* 10.4, CH***H***Br), 4.04 (1H, t, *J* 1.9, Ins 2-***H***), 3.90 (1H, d, *J* 10.4, CH***H***Br) ppm; MS (ESI^+) *m/z* (%) found $[M(^{79}Br)+H]^+$ 539 (98), $[M(^{81}Br)+H]^+$ 541 (100), $[M(^{79}Br)+Na]^+$ 561 (89), $[M(^{81}Br)+Na]^+$ 563 (90); HRMS (ESI^+) found $[M(^{79}Br)+H]^+$ 539.1085, $C_{28}H_{28}O_6{}^{79}Br$ requires 539.1069 and $[M(^{81}Br)+H]^+$ 541.1068, $C_{28}H_{28}O_6{}^{81}Br$ requires 541.1049.

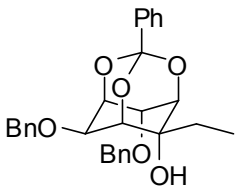
2,6-*O*-Dibenzyl-4-*C*-pentyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (133)



Alkyl lithium method on epoxide: 2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 170 mg, 0.38 mmol) was evaporated from toluene (3 × 2 mL) and taken up in ether (2 mL). *n*-Butyl lithium (1.6 M in hexanes, 476 μL, 0.76 mmol) was added drop-wise to the stirred solution. After 6 h the reaction was quenched with H_2O (1 mL), diluted with ether and the organic layer washed with H_2O (× 2), then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 1:4 v/v) afforded the *title compound* (**133**, 34 mg, 18 %) as a pale yellow oil.

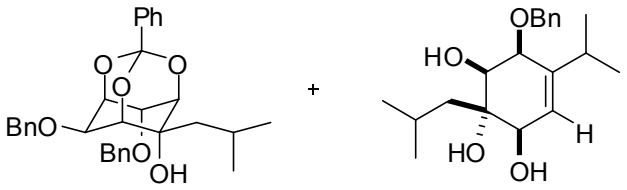
Alkyl lithium cuprates on epoxide, Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 335 mg, 0.72 mmol) was treated with butyl lithium cuprate according to general method 1. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:4 v/v) afforded the *title compound* (**133**, 338 mg, 90 %) as a pale yellow oil: R_f (EtOAc-hexane, 3:7 v/v) 0.61; δ_H (400 MHz, $CDCl_3$) 7.67-7.64 (2H, m), 7.47-7.44 (2H, m), 7.41-7.32 (9H, m), 7.24-7.22 (2H, m) (15 × Ar ***H***), 4.82 (1H, d, *J* 12.7), 4.70 (1H, d, *J* 12.7), 4.62 (1H, d, *J* 11.2), 4.57 (1H, d, *J* 11.2) (4 × OCH***H***Ph), 4.53 (1H, t, *J* 4.1, Ins 6-***H***), 4.48 (1H, dq, *J* 3.9, 2.0, Ins 1-***H***), 4.25 (1H, q, *J* 2.0, Ins 3-***H***), 4.23 (1H, bs, Ins 4-O***H***), 4.10 (1H, dt, *J* 3.9, 2.0, Ins 5-***H***), 4.01 (1H, t, *J* 2.0, Ins 2-***H***), 2.08 (1H, dt, *J* 13.2, 4.4, CH***H***$(CH_2)_3CH_3$), 1.95 (1H, dt, *J* 13.6, 4.8, CH***H***$(CH_2)_3CH_3$), 1.52-1.31 (6H, m, $CH_2(C\boldsymbol{H_2})_3CH_3$), 0.93 (3H, t, *J* 6.8, $CH_2(CH_2)C\boldsymbol{H_3}$) ppm; δ_C (125 MHz, $CDCl_3$) 137.92, 137.01, 135.90 (3 × Ar ***C***), 129.42, 128.83 (2C), 128.73, 128.50 (2C), 128.13 (2C), 127.97 (4C), 127.87, 125.36 (2C) (15 × Ar ***C***H), 107.28 (Ph***C***O_3), 75.82, 74.72 (2 × Ins ***C***H), 73.01, 71.08 (2 × O***C***H_2Ph), 70.97 (2 × Ins ***C***H), 70.92 (Ins ***C***), 66.37 (Ins ***C***H), 35.57, 32.17, 22.71, 21.62 (4 × ***C***H_2), 14.04 (***C***H_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 517.2598 (100), $C_{32}H_{37}O_6$ requires 517.2590.

2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (136)



2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 255 mg, 0.55 mmol) was treated with methyl lithium cuprate according to general method 1. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 3:7 v/v) afforded the *title compound* (**136**, 241 mg, 92 %) as a pale yellow oil; R_f (EtOAc-hexane, 3:7 v/v) 0.57; δ_H (400 MHz, $CDCl_3$) 7.69-7.67 (2H, m), 7.48-7.34 (11H, m), 7.25-7.23 (2H, m) (15 × Ar ***H***), 4.83 (1H, d, *J* 12.2), 4.71 (1H, d, *J* 12.2), 4.62 (1H, d, *J* 11.7), 4.57 (1H, d, *J* 11.7) (4 × OCH***H***Ph), 4.54 (1H, t, *J* 3.9, Ins 6-***H***), 4.49 (1H, dq, *J* 3.9, 2.0, Ins 1-***H***), 4.27 (1H, q, *J* 2.0, Ins 3-***H***), 4.23 (1H, bs, Ins 4-O***H***), 4.10 (1H, dt, *J* 3.4, 1.5, Ins 5-***H***), 4.03 (1H, t, *J* 1.5, Ins 2-***H***), 2.15 (1H, dq, *J* 14.0, 7.3, CH***H***CH_3), 2.01 (1H, dt, *J* 21.5, 7.3, CH***H***CH_3), 1.03 (3H, t, *J* 7.3, $CH_2C\boldsymbol{H_3}$) ppm; δ_C (125 MHz, $CDCl_3$) 137.91, 136.98, 135.91 (3 × Ar ***C***), 129.42, 128.84 (2C), 128.74, 128.50 (2C), 128.14 (2C), 127.97 (4C), 127.88, 125.35 (2C) (15 × Ar ***C***H), 107.27 (Ph***C***O_3), 75.56 (Ins 3-***C***H), 74.66 (Ins 6-***C***H), 73.02, 71.09 (2 × O***C***H_2Ph), 70.98 (Ins 1-***C***H), 70.88 (Ins 4-***C***), 70.62 (Ins 5-***C***H), 66.41 (Ins 2-***C***H), 28.27 (***C***H_2CH_3), 6.35 (CH_2***C***H_3) ppm; MS (ESI^+) *m/z* (%) found $[M+H]^+$ 475 (100), $[M+Na]^+$ 497 (82), $[2M+Na]^+$ 971 (53); HRMS (ESI^+) found $[M+H]^+$ 475.2137, $C_{29}H_{31}O_6$ requires 475.2121.

2,6-*O*-Dibenzyl-4-*C*-isobutyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (137) and 6-benzyloxy-2-isobutyl-5-isopropyl-cyclohex-4-ene-1,2,3-triol (138)



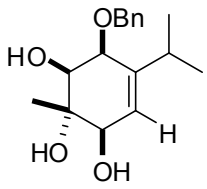
2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 170 mg, 0.37 mmol) was treated with *iso*-propyl lithium cuprate according to general method 1. The crude material was fractionated by chromatography on flash silica to yield two compounds – 2,6-*O*-dibenzyl-4-*C*-*iso*-butyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**137**, 48 mg, 26 %) as a pale yellow oil and 6-benzyloxy-2-isobutyl-5-isopropyl-cyclohex-4-ene-1,2,3-triol (**138**, 42 mg, 34 %) as a white crystalline solid.

For 2,6-*O*-dibenzyl-4-*C*-isobutyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **137**; R_f (EtOAc-hexane, 3:7 v/v) 0.60; δ_H (400 MHz, $CDCl_3$) 7.68-7.65 (2H, m), 7.48-7.45 (2H, m), 7.42-7.35 (9H, m), 7.25-7.23 (2H, m) (15 × Ar ***H***), 4.82 (1H, d, *J* 12.2), 4.71 (1H, d, *J* 12.7), 4.63 (1H, d, *J* 11.7), 4.58 (1H, d, *J* 11.2) (4 × OCH***H***Ph), 4.53 (1H, t, *J* 4.2, Ins 6-***H***), 4.49 (1H, dq, *J* 3.9, 1.5, Ins 1-***H***), 4.25 (1H, q, *J* 2.0, Ins 3-***H***), 4.21 (1H, s, Ins 4-O***H***), 4.13 (1H, dt, *J* 3.9, 2.0, Ins 5-***H***), 4.03 (1H, t, *J* 1.5, Ins 2-***H***), 2.08-1.96 [2H, m, CH***H***CH$(CH_3)_2$ + CH_2C***H***$(CH_3)_2$], 1.88 [1H, d, *J* 13.2, 5.4, CH***H***CH$(CH_3)_2$], 1.05 [3H, d, *J* 6.4, $CH_2C(\boldsymbol{CH_3})_2$], 1.04 [3H, d, *J* 6.4, $CH_2C(\boldsymbol{CH_3})_2$] ppm; δ_C (125 MHz, $CDCl_3$) 137.89, 137.02, 135.88 (3 × Ar ***C***), 129. 36, 128.80 (2C), 128.70, 128.48 (2C), 128.11 (2C), 127.95 (4C), 127.85, 125.34 (2C) (15 × Ar ***C***H), 107.23 (Ph***C***O_3), 76.09, 74.82 (2 × Ins ***C***H), 73.00 (O***C***H_2Ph), 71.44 (Ins ***C***), 71.08 (O***C***H_2Ph), 70.97, 70.81, 66.25 (3 × Ins ***C***H), 43.89 [***C***H_2CH$(CH_3)_2$], 24.58 [2 × CH_2CH(***C***$H_3)_2$], 23.04 [CH_2***C***H$(CH_3)_2$] ppm; MS (ESI^+) *m/z* (%) found $[M+H]^+$ 503 (93), $[M+Na]^+$ 525 (100); HRMS (ESI^+) 503.2440, $C_{31}H_{35}O_6$ requires 503.2434.

For 6-benzyloxy-2-isobutyl-5-isopropyl-cyclohex-4-ene-1,2,3-triol **138**; R_f (EtOAc-hexane, 3:7 v/v) 0.44; δ_H (400 MHz, $CDCl_3$) 7.40 -7.31 (5H, m, 5 × Ar ***H***), 5.74 (1H, d, *J* 5.4, Ins 6-***H***), 4.72 (1H, d, *J* 11.2), 4.62 (1H, d, *J* 10.8) (2 × OCH***H***Ph), 4.40 (1H, d, *J* 3.9, Ins 2-***H***), 4.08 (1H, dd, *J* 4.4, 2.0, Ins 3-***H***), 3.74 (1H, dd, *J* 11.7, 5.4, ex → d, Ins 5-***H***), 3.09 (1H, d, *J* 11.2, ex, Ins 5-O***H***), 2.86 (1H, s, ex, Ins 3-O***H***), 2.68 [1H, septet, *J* 6.8, 1-C***H***$(CH_3)_2$], 2.10 [1H, m, 4-CH_2C***H***$(CH_3)_2$], 2.03 [1H, dd, *J* 15.1, 5.4, 4-CH***H***CH$(CH_3)_2$], 1.71 [1H, dd, *J* 14.7, 7.3, 4-CH***H***CH$(CH_3)_2$], 1.12 [3H, d, *J* 6.8, 1-CH_2CH(C***H***$_3)_2$], 1.08 [3H, d, *J* 6.4, 4-CH_2CH(C***H***$_3)_2$], 1.06 [3H, d, *J* 6.4, 4-CH_2CH(C***H***$_3)_2$], 1.02 [3H, d, *J* 6.8, 1-CH_2CH(C***H***$_3)_2$] ppm; δ_C (125 MHz, $CDCl_3$) 142.50 (Ins 1-***C***) 137.49 (Ar ***C***), 128.56 (2C), 127.65, 126.97 (2C) (5 × Ar ***C***H), 121.57 (Ins 6-***C***H), 75.65 (Ins ***C***H), 73.86 (Ins ***C***), 72.17 (O***C***H_2Ph), 70.63, 70.60 (2 × Ins ***C***H), 44.50 [4-***C***H_2CH$(CH_3)_2$], 28.58 [1-***C***H$(CH_3)_2$], 25.04, 24.75 [2 × 4-CH(***C***$H_3)_2$], 22.69 [4-CH_2***C***H$(CH_3)_2$], 22.14, 20.56 [2 × 1-CH(***C***$H_3)_2$] ppm; MS (ESI^+) *m/z* (%) found $[M+Na]^+$ 357 (43),

$[M+Na+MeCN]^+$ 398 (58), $[2M+Na]^+$ 691 (100); HRMS (ESI^+) found $[M+Na]^+$ 357.2045, $C_{20}H_{30}O_4Na$ requires 357.2042.

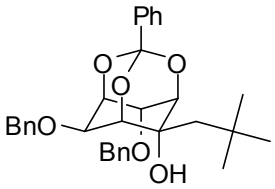
6-Benzyloxy-2-methyl-5-isopropyl-cyclohex-4-ene-1,2,3-triol (139)



2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**115**, 135 mg, 0.29 mmol) was treated with *iso*-propyl lithium cuprate according to general method 1. The crude material was fractionated by chromatography on flash silica to yield the *title compound* (**139**, 25 mg, 30 %) and starting material (**115**, 91 mg, 68 %). For the *title compound*; R_f (EtOAc-hexane, 3:7 v/v) 0.49; δ_H (400 MHz, $CDCl_3$) 7.41 -7.35 (5H, m, Ar ***H***), 5.68 (1H, d, *J* 4.2, Ins 6-***H***), 4.75 (1H, d, *J* 11.2), 4.67 (1H, d, *J* 11.2) (2 × OCH***H***Ph), 4.35 (1H, d, *J* 4.1, Ins 2-***H***), 3.98 (1H, d, *J* 3.9, Ins 3-***H***), 3.76 (1H, bs, Ins 5-***H***), 2.86 (1H, bs, ex, Ins 5-O***H***), 2.82 (1H, s, ex, Ins 3-O***H***), 2.63 [1H, septet, *J* 6.8, 1-C***H***$(CH_3)_2$], 1.50 (3H, s, 4-C***H₃***), 1.13 [3H, d, *J* 6.7, 1-CH(C***H₃***$)_2$], 1.04 [3H, d, *J* 6.8, 1-CH(C***H₃***$)_2$] ppm; δ_C (100 MHz, $CDCl_3$) 142.97 (Ins 1-***C***), 137.65 (Ar ***C***), 128.63 (2C), 128.12, 127.82 (2C) (5 × Ar ***C***H), 122.17 (Ins 6-***C***H), 75.86 (Ins 2-***C***H), 73.11 (Ins 5-***C***H), 72.91 (O***C***H_2Ph), 72.64 (Ins 4-***C***), 72.15 (Ins 3-***C***H), 29.02 [1-***C***H$(CH_3)_2$], 22.61 (4-***C***H_3), 22.26, 20.81 [2 × 1-CH(***C***$H_3)_2$] ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 315.1580 (42), $C_{17}H_{24}O_4Na$ requires 315.1572.

2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate, **115**, as characterised previously.

2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (144)

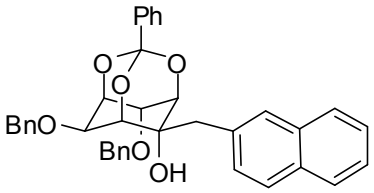


2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 400 mg, 0.87 mmol) was treated with *tert*-butyl lithium cuprate according to general method 1. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:4 v/v) afforded the *title compound* (**144**, 161 mg, 36 %) as a pale yellow oil and the reduction product 2,6-*O*-dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**115**, 121 mg, 30 %) as a clear oil.

For the title compound **144**; R_f (EtOAc-hexane, 3:7 v/v) 0.62; δ_H (400 MHz, $CDCl_3$) 7.68-7.65 (2H, m), 7.47-7.45 (2H, m), 7.41-7.34 (9H, m), 7.25-7.23 (2H, m) (15 × Ar ***H***), 4.80 (1H, d, *J* 12.7), 4.70 (1H, d, *J* 12.7), 4.62 (1H, d, *J* 11.2), 4.58 (1H, d, *J* 11.2) (4 × OCH***H***Ph), 4.52 (1H, t, *J* 4.2, Ins 6-***H***), 4.46 (1H, dq, *J* 3.9, 2.0, Ins 1-***H***), 4.30 (1H, q, *J* 2.0, Ins 3-***H***), 4.25 (1H, dt, *J* 3.9, 2.0, Ins 5-***H***), 4.19 (1H, bs, Ins 4-O***H***), 4.02 (1H, t, *J* 2.0, Ins 2-***H***), 2.10 [1H, d, *J* 14.2, CH***H***C$(CH_3)_3$], 1.96 [1H, d, *J* 14.2, CH***H***C$(CH_3)_3$], 1.11 [9H, s, $CH_2C(\boldsymbol{CH_3})_3$] ppm; δ_C (125 MHz, $CDCl_3$) 137.98, 137.11, 135.93 (3 × Ar ***C***), 129. 36, 128.83 (2C), 128.72, 128.48 (2C), 128.14 (2C), 127.95 (4C), 127.84, 125.40 (2C) (15 × Ar ***C***H), 107.22 (Ph***C***O_3), 76.89, 75.10 (2 × Ins ***C***H), 73.08 (O***C***H_2Ph), 72.30 (Ins ***C***), 71.54 (Ins ***C***H), 71.14 (O***C***H_2Ph), 70.66, 66.29 (2 × Ins ***C***H), 47.32 [***C***$H_2C(CH_3)_3$], 31.62 [$CH_2C(\boldsymbol{C}H_3)_3$], 31.23 [$CH_2\boldsymbol{C}(CH_3)_3$] ppm; HRMS ($ESI^+$) *m/z* (%) found $[M+H]^+$ 517.2590 (100), $C_{32}H_{37}O_6$ requires 517.2590.

2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate, **115**, as characterised previously.

2,6-*O*-Dibenzyl-4-*C*-(2-naphthyl)methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (145)



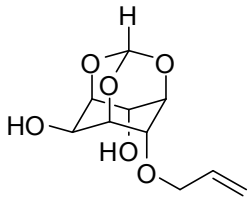
2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**98**, 335 mg, 0.73 mmol) was treated with napthyl lithium cuprate prepared according to general method 1, using 2-lithium napthalene, prepared from 2-bromo napthalene according to the following method; 2-bromo napthalene (1.36 mg, 6.55 mmol) was evaporated from toluene (2 × 5 mL) and taken up in ether-toluene (1:1 v/v, 8 mL). To this solution was added drop-wise, *n*-butyl lithium (1.0 M solution in hexanes, 6.50 mL, 6.55 mmol) over 5 min and the resulting solution stirred for 20 min, during which time the colour changed from cloudy white to pale orange.

The crude material after treatment with napthyl lithium cuprate was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 3:7 v/v) afforded the *title compound* (**145**, 283 mg, 66 %) as a pale yellow oil; R_f (EtOAc-hexane, 3:7 v/v) 0.41; δ_H (400 MHz, $CDCl_3$) 7.85-7.80 (6H, m), 7.50-7.25 (14H, m), 7.13-7.11 (2H, m) (22 × Ar ***H***), 4.77 (1H, d, *J* 12.2), 4.64 (1H, d, *J* 12.2), 4.50 (1H, d, *J* 12.2) (3 × OCH***H***Ph), 4.48-4.47 (2H, m, Ins 1-***H*** + Ins 6-***H***), 4.46 (1H, d, *J* 11.2, OCH***H***Ph), 4.27 (1H, q, *J* 1.5, Ins 3-***H***), 4.25 (1H, s, Ins 4-O***H***), 4.01 (1H, t, *J* 2.0, Ins 2-***H***), 3.96 (1H, dt, *J* 3.4, 1.4, Ins 5-***H***), 3.60 (1H, d, *J* 13.7), 3.40 (1H, d, *J* 13.7) (2 × CH***H***Ar) ppm; δ_C (100 MHz, $CDCl_3$) 137.87, 137.11, 135.75, 133.66, 133.39, 132.42 (6 × Ar ***C***), 129.70, 129.59 (2C), 128.78 (2C), 128.71, 128.52 (2C), 128.19 (2C), 128.14 (2C), 128.02 (2C), 127.91, 127.85, 127.58, 127.39, 125.76, 125.52 (2C), 125.43 (22 × Ar ***C***H), 107.54 (Ph***C***O_3), 75.75,

74.66 (2 × Ins ***C***H), 73.18 (O***C***H_2Ph), 71.35 (Ins ***C***), 71.22 (O***C***H_2Ph), 70.95, 70.48, 66.40 (3 × Ins ***C***H), 41.78 (4-C***C***H_2Ar) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 587 (54), $[M+Na]^+$ 609 (100); HRMS (ESI^+) *m/z* found $[M+H]^+$ 587.2428, $C_{38}H_{35}O_6$ requires 587.2434.

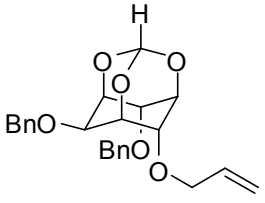
7.4 Chapter 4

4-*O*-Allyl-*myo*-inositol 1,3,5-orthoformate (31)[76]



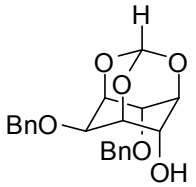
To m*yo*-inositol (10.80 g, 60.0 mmol) in DMF (150 mL) was added trimethylorthoformate (18.0 mL, 175 mmol) and *p*-toluene sulfonic acid (3.00g, 16 mmol). The clear solution was stirred at 100 ºC for 18 h before addition of triethyl amine (to pH 7) followed by evaporation of all solvents under reduced pressure. The resulting off-white solid was evaporated from MeCN (3 × 15 mL) and taken up in DMF (100 mL). After cooling to -15 ºC, NaH (60 % dispersion in mineral oil, 2.40g, 60 mmol) was added portion-wise and the solution allowed to warm to rt for 30 min. Allyl bromide (5.2 mL, 60 mmol) was added drop-wise and the solution stirred for 12h. The DMF was reduced under high vacuum and the resulting gum taken up in EtOAc, washed with H_2O (× 3), then brine, dried ($MgSO_4$) and all solvents evaporated. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 4:6 v/v) afforded the *title compound* (**31**, 4.70 g, 34 %) as a pale yellow oil; R_f (EtOAc-hexane 1:1 v/v) 0.19; δ_H (400 MHz, $CDCl_3$) 5.88 (1H, ddt, *J* 17.0, 10.5, 5.9, $OCH_2C\boldsymbol{H}CH_2$), 5.48 (1H, s, $O_3C\boldsymbol{H}$), 5.34 (1H, dq, *J* 17.1, 1.0, $OCH_2CHCH\boldsymbol{H}$), 5.31 (1H, dq, *J* 10.2, 1.0, $OCH_2CHCH\boldsymbol{H}$), 4.48 (1H, bs, Ins 6-***H***), 4.39 (1H, td, *J* 3.9, 1.5, Ins 4-***H***), 4.33 (1H, dq, *J* 3.4, 2.0, Ins 3-***H***), 4.31 (1H, tt, *J* 3.9, 2.0, Ins 5-***H***), 4.23 (1H, dq, *J* 3.9, 2.0, Ins 1-***H***), 4.22-4.14 (2H, m, $OC\boldsymbol{H_2}CHCH_2$), 4.08 (1H, bs, Ins 2-***H***), 3.76 (1H, bd, *J* 8.84, Ins-OH), 3.34 (1H, bs, Ins-OH) ppm; δ_C (100 MHz, $CDCl_3$) 132.65 ($OCH_2\boldsymbol{C}HCH_2$), 119.54 ($OCH_2CH\boldsymbol{C}H_2$), 102.71 ($O_3\boldsymbol{C}H$), 74.74, 74.18, 72.24 (3 × Ins ***C***H), 71.96 (O***C***H_2CHCH$_2$), 67.83, 67.26, 60.64 (3 × Ins ***C***H) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 231 (47), $[M+NH_4]^+$ 248 (100); HRMS (CI^+) found 248.1139, $C_{10}H_{18}NO_6$ requires 248.1134.

2,6-*O*-Dibenzyl-4-*O*-allyl-*myo*-inositol 1,3,5-orthoformate (32)[76]



4-*O*-Allyl-*myo*-inositol 1,3,5-orthoformate (4.70 g, 20.4 mmol) was evaporated from MeCN (3 × 10 mL), taken up in DMF (50 mL) and cooled to -15 °C. Sodium hydride (60% dispersion in mineral oil, 2.45 g, 61.28 mmol) was added portion-wise, the reaction stirred at -15 °C for 30 min then warmed to rt and benzyl bromide (7.29 mL, 61.28 mmol) was added drop-wise. The reaction was stirred at 60 °C for 12 h then quenched by drop-wise addition of H_2O (5 mL) and stirred for a further 30 min. The solvent volume was reduced under high vacuum, the residue dissolved in EtOAc and washed with H_2O (× 3) then brine. The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica in a large sinter funnel. Elution with EtOAc-hexane (1:9 → 3:7 v/v) afforded the *title compound* (8.03 g, 96 %) as a pale yellow oil; R_f (EtOAc-hexane 1:1 v/v) 0.65, δ_H (400 MHz, $CDCl_3$) 7.42-7.28 (10H, m, 10 × Ar ***H***), 5.83 (1H, ddt, *J* 17.1, 10.5, 5.5, $OCH_2C\boldsymbol{H}CH_2$), 5.55 (1H, s, $O_3C\boldsymbol{H}$), 5.24 (1H, dq *J* 17.2, 1.4, $OCH_2CHCH\boldsymbol{H}$), 5.17 (1H, dq *J* 10.5, 0.8, $OCH_2CHCH\boldsymbol{H}$), 4.70 (2H, s, $OC\boldsymbol{H}_2Ph$), 4.64 (1H, d, *J* 11.8, $OCH\boldsymbol{H}Ph$), 4.50 (1H, d, *J* 11.8, $OCH\boldsymbol{H}Ph$), 4.42 (1H, m, Ins ***H***), 4.34-4.31 (2H, m, 2 × Ins ***H***), 4.30-4.26 (2H, m, 2 × Ins ***H***), 4.09 (1H, ddt, *J* 12.4, 5.2, 1.0, $OCH\boldsymbol{H}CHCH_2$), 4.03 (1H, q, *J* 1.2, Ins 2-***H***), 3.99 (1H, ddt, *J* 12.8, 5.6, 1.2, $OCH\boldsymbol{H}CHCH_2$) ppm; δ_C (100 MHz, $CDCl_3$) 137.89, 137.73 (2 × Ar ***C***), 134.10 ($OCH_2\boldsymbol{C}HCH_2$), 128.46 (2C), 128.43 (2C), 128.11 (2C), 127.88 (2C), 127.53 (2C) (10 × Ar ***C***H), 117.54 ($OCH_2CH\boldsymbol{C}H_2$), 103.25 ($O_3\boldsymbol{C}H$), 73.93, 73.85 (2 × Ins ***C***H), 71.67, 71.45 (2 × OCH_2Ph), 70.72 (Ins ***C***H), 70.66 ($O\boldsymbol{C}H_2CHCH_2$), 70.59, 68.16, 67.47 (3 × Ins ***C***H) ppm; MS (ESI^+) *m/z* (%) found $[M+H]^+$ 411 (62), $[M+Na]^+$ 433 (67); HRMS (ESI^+) found 411.1812, $C_{24}H_{27}O_6$ requires 411.1808.

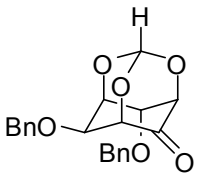
2,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-orthoformate (41)[90]



2,6-*O*-Dibenzyl-4-*O*-(prop-1-enyl)-*myo*-inositol 1,3,5-*O*-orthoformate (**159**, 4.60 g, 11.21 mmol) was taken up in MeOH (250 mL) and NaOMe (25 mL). A solution of $KMnO_4$ (1.95 g, 12.34 mmol) in H_2O (250 mL) was added drop-wise over 30 min and the resulting solution stirred for a further 30 min during which time a solid brown precipitate formed. The mixture was filtered under gravity and the mother liquor neutralised with HCl (4 M). The volume was reduced under reduced pressure and the crude material extracted into EtOAc (× 2). The combined organic layers were washed with brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica in a large sinter funnel. Elution with hexane-EtOAc (1:0 → 1:1 v/v) afforded the *title compound* (**41**, 3.41 g, 82 %) as a colourless

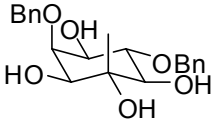
oil; R_f (hexane-EtOAc 4:1 v/v) 0.12; δ_H (400 MHz, $CDCl_3$) 7.43-7.35 (8H, m), 7.22-7.20 (2H, m) (10 × Ar ***H***), 5.54 (1H, s, O_3C***H***), 4.78 (1H, d, *J* 12.3), 4.69 (1H, d, *J* 12.3), 4.57 (1H, d, *J* 11.5), 4.52 (1H, d, *J* 11.6) (4 × OCH***H***Ph), 4.50-4.48 (1H, m, Ins 4-***H***), 4.41-4.37 (2H, m, 2 × Ins ***H***), 4.30-4.28 (2H, m, 2 × Ins ***H***), 3.91 (1H, q, *J* 1.0 Ins 2-***H***), 3.64 (1H, bd, *J* 10.0, Ins 4-O***H***) ppm; δ_C (100 MHz, $CDCl_3$) 137.58, 135.92 (2 × Ar ***C***), 128.89 (2C), 128.82, 128.63 (2C), 128.12 (2C), 128.08 (3C) (10 × Ar ***C***H), 102.66 (O_3***C***H), 74.52 (Ins C***H***), 72.97 (O***C***H_2Ph), 72.19 (Ins ***C***H), 71.42 (O***C***H_2Ph), 69.99, 68.03, 67.69, 66.16 (4 × Ins ***C***H) ppm; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 371.1499 (46), $C_{21}H_{23}O_6$ requires 371.1495.

2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-orthoformate (42)[90]



2,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthoformate (**41**, 1.21 g, 3.28 mmol) was evaporated from MeCN (3 × 5 mL) and taken up in CH_2Cl_2 (10 mL). Dess Martin periodinane (2.78 g, 6.56 mmol) was added portion-wise and the suspension stirred for 3 h. $Na_2S_2O_3.H_2O$ (10 % solution in H_2O) was added and stirring continued for 10 min. The product was extracted with CH_2Cl_2 (× 2), and the combined organic layers washed successively with sat. $NaHCO_3$, H_2O and brine, before drying ($MgSO_4$) and evaporation to dryness under reduced pressure. The crude inos-4-ose (1.15 g, 96 %) was used without further purification; R_f (hexane-EtOAc, 1:1 v/v) 0.22; δ_H (400 MHz, $CDCl_3$) 7.42-7.36 (8H, m), 7.23-7.21 (2H, m) (10 × Ar ***H***), 5.71 (1H, s, O_3C***H***), 4.76 (1H, d, *J* 12.1), 4.65 (1H, d, *J* 12.1), 4.59 (1H, d, *J* 11.8) (3 × OCH***H***Ph), 4.51 (1H, q, *J* 1.7, Ins 3-***H***), 4.44 (1H, d, *J* 11.7, OCH***H***Ph), 4.46-4.44 (1H, m, Ins ***H***), 4.39-4.36 (2H, m, 2 × Ins ***H***), 3.79 (1H, q *J* 1.4, Ins 2-***H***) ppm; δ_C (100 MHz, $CDCl_3$) 199.30 (Ins ***C***=O), 136.96, 136.37 (2 × Ar ***C***), 128.71 (2C), 128.68 (2C), 128.50, 128.26, 128.04 (2C), 127.94 (2C) (10 × Ar ***C***H), 103.00 (O_3***C***H), 78.97, 76.81 (2 × Ins ***C***H), 71.86, 71.75 (2 × O***C***H_2Ph), 71.33, 70.61, 70.53 (3 × Ins ***C***H) ppm; MS (ESI^+) *m/z* (%) found $[M+H]^+$ 369 (52), $[M+H_3O]^+$ 387 (100); HRMS (ESI^+) found $[M+H]^+$ 369.1347, $C_{21}H_{21}O_6$ requires 369.1338.

2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol (151a)

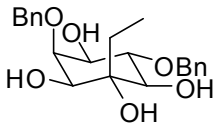


2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**115**, 200 mg, 0.43 mmol) was refluxed in conc. HCl-methanol (6 mL, 1:2 v/v). After 3 h the solution was cooled, diluted

with H_2O and extracted with EtOAc. The organic layer was washed with sat. $NaHCO_3$, H_2O, and then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. ^1H NMR illustrated that more than one isomeric product was present, including **150a**. If desired **150a** may be isolated by chromatography on flash silica, eluting with EtOAc-hexane (1:4 → 3:2 v/v); δ_H (400 MHz, CD_3OD) 7.93-7.06 (15H, m, 15 × Ar ***H***), 5.11 (1H, dd, *J* 10.1, 3.3, Ins 1-***H***), 4.84 (1H, d, *J* 11.1), 4.81 (1H, d, *J* 11.7), 4.68 (1H, d, *J* 11.1), 4.59 (1H, d, *J* 11.7) (4 × PhCH***H***O), 4.10 (1H, t, *J* 3.1, Ins 2-***H***), 3.90 (1H, t, *J* 10.0, Ins 6-***H***), 3.67 (1H, d, *J* 3.0, Ins 3-***H***), 3.52 (1H, d, *J* 9.8, Ins 5-***H***), 1.41 (3H, s, Ins 4-C***H₃***) ppm; δ_C (100 MHz, CD_3OD) 167.26 (Ph***C***O_2), 140.13, 139.80 (2 × Ar ***C***), 134.41 (2 × Ar ***C***H), 131.13 (Ar ***C***), 130.80 (3C), 129.57 (2C), 129.17, 129.08 (3C), 128.55 (2C), 128.47, 128.36 (13 × Ar ***C***H), 80.21, 79.86, 79.12 (3 × Ins ***C***H), 77.70 (Ins 4-***C***CH_3), 76.40, 76.17 (2 × Ph***C***H_2O), 75.56, 75.30 (2 × Ins ***C***H), 16.97 (Ins 4-C***C***H_3); HRMS (CI^+) *m/z* (%) found $[M+NH_4]^+$ 496.2330 (100), $C_{28}H_{34}NO_7$ requires 496.2335.

The crude mixture of isomeric benzoates **150a-c** (180 mg, 0.38 mmol) was evaporated from MeCN (3 × 1 mL) and taken up in MeOH (2 mL). NaOMe (25 % solution in MeOH, 52 µL, 0.19 mmol) was added and the reaction refluxed for 3 h. After careful neutralisation with 4 M HCl all solvents were evaporated under reduced pressure. The residue was taken up in methanol, the solids removed by filtration, and the mother-liquor evaporated to dryness. The crude material was fractionated by chromatography on flash silica. Elution with CH_2Cl_2-hexane (0:1 → 1:0 v/v) then EtOH-CH_2Cl_2 (0:1 → 3:97 v/v) afforded the *title compound* (**151a**, 139 mg, 87 %) as a colourless glass; δ_H (400 MHz, CD_3OD) 7.46-7.25 (10H, m, 10 × Ar ***H***), 4.89 (1H, d, *J* 11.3) 4.85-4.80 (3H, m) (4 × OCH***H***Ph), 3.90 (1H, t, *J* 3.1, Ins 2-***H***), 3.67 (1H, dd, *J* 9.6, 3.2, Ins 1-***H***), 3.60-3.55 (2H, m, Ins 6-***H*** + Ins 3-***H***), 3.43 (1H, d, *J* 9.4, Ins 5-***H)***, 1.36 (3H, s, Ins 4-C***H₃***) ppm; MS (ESI^+) *m/z* (%) found $[M+Na]^+$ 397.1628 (100), $C_{21}H_{26}O_6Na$ requires 397.1627.

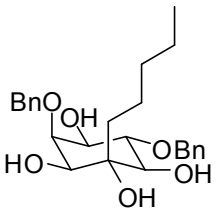
2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol (151b)



2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**136**, 280 mg, 0.60 mmol) was refluxed in conc. HCl-(propan-1-ol) according to general method 4 to recover a crude mixture of benzoyls (294 mg), HRMS (ESI^+) *m/z* (%) found [M+H] 493.2246 (32), $C_{29}H_{33}O_7$ requires 493.2226, $[M+Na]^+$ 515.2055 (100), $C_{29}H_{32}NaO_7$ requires 515.2046. The crude mixture was treated with NaOMe in MeOH according to general method 5 to afford the *title compound* (**151b**, 204 mg, 88 % over 2 steps) as a pale yellow oil; R_f (EtOAc-hexane 7:3 v/v) 0.20; δ_H (500 MHz, $CDCl_3$) 7.38-7.32 (8H, m), 7.26-7.24 (2H, m) (10 × Ar ***H***), 4.72 (1H, d, *J* 11.5), 4.67 (1H, d, *J* 11.5), 4.65 (1H, d, *J* 11.6), 4.59 (1H, d, *J* 11.6) (4 × OCH***H***Ph), 4.28 (1H, bs, Ins 1-***H***), 4.13 (1H, t,

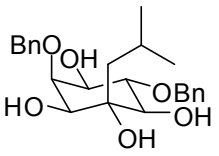
J 3.0, Ins 2-***H***), 4.04 (1H, bs, Ins 5-***H***), 3.98 (1H, t, *J* 3.4, Ins 6-***H***), 3.80 (1H, bs, Ins 4-O***H***), 3.73 (1H, bd, *J* 8.1, Ins 3-***H***), 3.32 (1H, bd, *J* 10.6, Ins 3-O***H***), 2.95 (1H, bs, Ins 1-O***H***), 2.74 (1H, bs, Ins 5-O***H***), 2.01 (1H, dq, *J* 14.4, 7.2), 1.85 (1H, dq, *J* 14.3, 7.0) (2 × CH***H***CH_3), 1.02 (3H, t, *J* 7.4, CH_2C***H₃***) ppm; δ_C (125 MHz, $CDCl_3$) 137.44, 136.56 (2 × Ar ***C***), 128.70 (2C), 128.66 (2C), 128.41, 128.25, 128.08 (2C), 127.93 (2C) (10 × Ar ***C***H), 80.12 (Ins ***C***H), 74.99 (Ins ***C***), 74.10 (Ins ***C***H), 73.41 (O***C***HPh), 73.30 (Ins ***C***H), 71.32 (O***C***HPh), 70.86, 69.71 (2 × Ins ***C***H), 27.68 (***C***H_2CH_3), 6.12 (CH_2***C***H_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 411.1799, $C_{22}H_{28}O_6Na$ requires 411.1784.

2,6-*O*-Dibenzyl-4-*C*-pentyl-*myo*-inositol (151c)



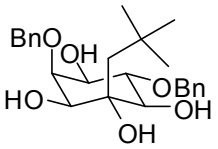
2,6-*O*-Dibenzyl-4-*C*-pentyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**133**, 170 mg, 0.34 mmol) was refluxed in conc. HCl-(propan-1-ol) according to general method 4, to recover a crude mixture of benzoyls (175 mg); HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 557.2525 (100), $C_{32}H_{38}O_7Na$ requires 557.2515. The crude mixture was treated with NaOMe in MeOH according to general method 5 to afford the *title compound* (**151c**, 71 mg, 49 %) as a pale yellow oil; R_f (EtOAc) 0.28; δ_H (400 MHz, $CDCl_3$) 7.43-7.33 (8H, m), 7.29-7.26 (2H, m) (10 × Ar ***H***), 4.76 (1H, d, *J* 11.2), 4.71 (1H, d, *J* 11.2), 4.69 (1H, d, *J* 11.7), 4.62 (1H, d, *J* 11.2) (4 × OCH***H***Ph), 4.32 (1H, m, Ins 1-***H***), 4.16 (1H, t, *J* 2.9, Ins 2-***H***), 4.08 (1H, m, Ins 5-***H***), 4.02 (1H, t, *J* 3.4, Ins 6-***H***), 3.85 (1H, bs, ex, Ins O***H***), 3.78 (1H, bd, *J* 4.9, ex → bs, Ins 3-***H***), 3.41 (1H, bd, *J* 8.3, ex, Ins O***H***), 3.16 (1H, bs, ex, Ins O***H***), 2.92 (1H, bs, ex, Ins O***H***), 1.99-1.92 (1H, m), 1.89-1.81 (1H, m) [2 × CH***H***$(CH_2)_3CH_3$], 1.55-1.51 [2H, m, CH_2(CH_2***H₂***$)_3CH_3$], 1.42-1.31 [4H, m, 2 × CH_2(CH_2***H₂***$)_3CH_3$], 0.94 [3H, t, *J* 6.8, $CH_2(CH_2)_3C$***H₃***] ppm; δ_C (100 MHz, $CDCl_3$) 137.43, 136.57 (2 × Ar ***C***), 128.33 (2C), 128.49 (2C), 128.44, 128.27, 128.12 (2C), 127.96 (2C) (10 × Ar ***C***H), 80.12 (Ins ***C***H), 75.08 (Ins ***C***), 74.36 (Ins ***C***H), 73.44 (O***C***H_2Ph), 73.29 (Ins ***C***H), 71.36 (O***C***H_2Ph), 71.20, 69.75 (2 × Ins ***C***H), 35.08, 32.42, 22.78, 21.37 (4 × ***C***H_2), 14.13 (***C***H_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 453.2254, $C_{25}H_{34}O_6Na$ requires 453.2253.

2,6-*O*-Dibenzyl-4-*C*-*iso*-butyl-*myo*-inositol (151d)



2,6-*O*-Dibenzyl-4-*C*-*iso*-butyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**137**, 171 mg, 0.34 mmol) was refluxed with conc. HCl-(propan-1-ol) according to general method 4, to recover a crude mixture of benzoyls (120 mg); HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 543.2387 (100), $C_{31}H_{36}O_7Na$ requires 543.2359. The crude mixture was treated with NaOMe in MeOH according to general method 5 to afford the *title compound* (**151d**, 28 mg, 20 %) as a yellow oil; R_f (EtOAc-hexane 7:3 v/v) 0.21; δ_H (400 MHz, $CDCl_3$) 7.41-7.34 (8H, m), 7.27-7.25 (2H, m) (10 × Ar ***H***), 4.73 (1H, d, *J* 11.7), 4.69 (1H, d, *J* 11.7), 4.68 (1H, d, *J* 11.7), 4.60 (1H, d, *J* 11.7) (4 × OCH***H***Ph), 4.30 (1H, bs, Ins 1-***H***), 4.15 (1H, t, *J* 2.9, Ins 2-***H***), 4.08 (1H, bs, Ins 5-***H***), 4.01 (1H, t, *J* 3.4, Ins 6-***H***), 3.86 (1H, bs, Ins 4-O***H***), 3.78 (1H, bd, *J* 7.3, Ins 3-***H***), 3.40 (1H, bd, *J* 9.3, Ins 3-O***H***), 2.99 (1H, bs, Ins 1-O***H***), 2.78 (1H, bs, Ins 5-O***H***), 2.03 [1H, m, $CH_2C\boldsymbol{H}(CH_3)_2$], 1.89 [1H, dd, *J* 14.2, 5.4, $CH\boldsymbol{H}CH(CH_3)_2$], 1.76 [1H, dd, *J* 14.7, 6.4, $CH\boldsymbol{H}CH(CH_3)_2$], 1.05 [3H, s, $CH_2CH(C\boldsymbol{H_3})_2$], 1.03 [3H, s, $CH_2CH(C\boldsymbol{H_3})_2$] ppm; δ_C (125 MHz, $CDCl_3$) 137.44, 136.55 (2 × Ar ***C***), 128.71 (2C), 128.69 (2C), 128.43, 128.28, 128.12 (2C), 127.96 (2C) (10 × Ar ***C***H), 80.27 (Ins ***C***H), 75.72 (Ins ***C***), 74.65 (Ins ***C***H), 73.41 (O***C***H_2Ph), 73.14 (Ins ***C***H), 71.28 (O***C***H_2Ph), 71.19, 69.56 (2 × Ins ***C***H), 43.25 [***C***$H_2CH(CH_3)_2$], 24.90 [$CH_2CH(\boldsymbol{C}H_3)_2$], 22.75 [2 × $CH_2\boldsymbol{C}H(CH_3)_2$] ppm; HRMS ($ESI^+$) *m/z* (%) found $[M+Na]^+$ 439.2112 (100), $C_{24}H_{32}O_6Na$ requires 439.2097.

2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol (151e)

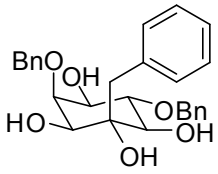


By acidic hydrolysis of the orthoester: 2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**144**, 98 mg, 0.19 mmol) was refluxed with conc. HCl-(propan-1-ol) according to general method 4, to recover a crude mixture of benzoyls (90 mg); HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 557.2520 (100), $C_{32}H_{38}O_7Na$ requires 557.2515. The crude mixture was treated with NaOMe in MeOH according to general method 5 to afford the *title compound* (**151e**, 40 mg, 49 %) as a clear oil.

By acidic hydrolysis of the benzylidene acetal: 2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol 3,5-*O*-benzylidene acetal (**176e**, 138 mg, 0.27 mmol) was taken up in CH_2Cl_2-EtOH-H_2O (5:1:1 v/v, 1.4 mL) and *p*-TsOH (10 mg, 0.05 mmol) added. After 24 h, all solvents were evaporated and the crude material purified by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 4:1 v/v) afforded the *title compound* (**151e**, 115 mg, 56 %) as a clear glass; R_f (EtOAc-hexane 7:3) 0.23; δ_H (400 MHz, $CDCl_3$) 7.41-7.35 (8H, m), 7.29-7.26 (2H, m) (10 × Ar ***H***), 4.72 (1H, d, *J* 11.7), 4.69 (1H, d, *J* 11.7), 4.67 (1H, d, *J* 11.7), 4.60 (1H, d, *J* 11.7) (4 × OCH***H***Ph), 4.29 (1H, bs, Ins 1-***H***), 4.16 (1H, t, *J* 2.9, Ins 2-***H***), 4.13 (1H, bs, Ins 5-***H***), 4.01 (1H, t, *J* 3.4, Ins 6-***H***), 3.89 (1H, bd, *J* 5.4, Ins 3-***H***), 3.87 (1H, bs, Ins 4-O***H***), 3.48 (1H, bd, *J* 6.8, Ins 3-

O***H***), 3.07 (1H, bs, Ins 1-O***H***), 2.85 (1H, bs, Ins 5-O***H***), 1.98 [1H, d, *J* 14.7, CH***H***C(CH$_3$)$_3$], 1.85 [1H, d, *J* 15.2, CH***H***C(CH$_3$)$_3$], 1.11 [9H, s, CH$_2$C(C***H$_3$***)$_3$] ppm; δ_C (125 MHz, CDCl$_3$) 137.45, 136.51 (2 × Ar ***C***), 128.69 (2C), 128.64 (2C), 128.40, 128.22, 128.07 (2C), 127.94 (2C) (10 × Ar ***C***H), 80.48 (Ins 2-***C***H), 76.60 (Ins 4-***C***), 75.66 (Ins 5-***C***H), 73.40 (O***C***H$_2$Ph), 72.99, 71.74 (Ins 6-***C***H + Ins 3-***C***H), 71.19 (O***C***H$_2$Ph), 69.19 (Ins 1-***C***H), 46.23 [***C***H$_2$C(CH$_3$)$_3$], 31.87 [CH$_2$C(***C***H$_3$)$_3$], 31.32 [CH$_2$***C***(CH$_3$)$_3$] ppm; HRMS (ESI$^+$) found [M+Na]$^+$ 453.2234, $C_{25}H_{34}O_6Na$ requires 453.2253.

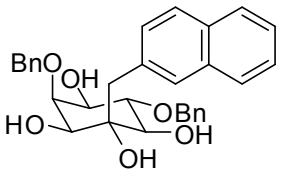
2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol (151f)



By acidic hydrolysis of the orthobenzoate: 2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**125**, 561 mg, 1.05 mmol) was refluxed in conc. HCl-(propan-1-ol) according to general method 4, to recover a crude mixture of benzoyls (580 mg); HRMS (ESI$^+$) *m/z* (%) found [M+H]$^+$ 555.2408, [M+Na]$^+$ 577.2216 (100), $C_{34}H_{35}O_7$ requires 555.2383. The crude mixture was treated with NaOMe in MeOH according to general method 5 to afford the *title compound* (**151f**, 195 mg, 41 % over two steps) as a pale yellow oil.

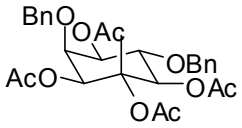
By acidic hydrolysis of the benzylidene acetal: 2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 3,5-*O*-benzylidene acetal (**176f**, 160 mg, 0.30 mmol) was taken up in CH$_2$Cl$_2$-EtOH-H$_2$O (5:1:1 v/v, 4 mL) and *p*-TsOH (11 mg, 0.06 mmol) added. After 24 h, all solvents were evaporated and the crude material purified by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 4:1 v/v) afforded the *title compound* (**151f**, 101 mg, 75 %) as a clear glass; R_f (EtOAc-hexane 7:3 v/v) 0.23; δ_H (400 MHz, CDCl$_3$) 7.43-7.26 (13H, m), 7.20-7.18 (2H, m) (15 × Ar ***H***), 4.67 (1H, d, *J* 11.2), 4.63 (1H, d, *J* 10.3), 4.60 (1H, d, *J* 11.2), 4.52 (1H, d, *J* 11.7) (4 × OCH***H***Ph), 4.27 (1H, bs, Ins 1-***H***), 4.15 (1H, t, *J* 2.9, Ins 2-***H***), 3.98-3.96 (2H, m, Ins 5-***H*** + Ins 6-***H***), 3.83 (1H, bs, Ins O***H***), 3.64 (1H, bs, Ins 3-***H***), 3.58 (1H, bs, Ins O***H***), 3.25 (1H, d, *J* 14.2), 3.21 (1H, d, *J* 14.2) (2 × 4-CH***H***Ph), 2.96 (1H, bs, Ins O***H***), 2.87 (1H, bs, Ins O***H***) ppm; δ_C (125 MHz, CDCl$_3$) 137.35, 136.48, 136.42 (3 × Ar ***C***), 131.20 (2C), 128.63 (2C), 128.61 (2C), 128.32, 128.24, 128.09 (2C), 127.87 (2C), 127.83 (2C), 126.23 (15 × Ar ***C***H), 80.14 (Ins ***C***H), 75.17 (Ins ***C***), 73.80, 73.35 (2 × Ins ***C***H), 73.34, 71.39 (2 × O***C***HPh), 71.21, 69.53 (2 × Ins ***C***H), 41.05 (4-***C***HPh) ppm; MS (CI$^+$) *m/z* (%) found [M+NH$_4$]$^+$ 468 (100); HRMS (CI$^+$) found [M+NH$_4$]$^+$ 468.2393, $C_{27}H_{34}NO_6$ requires 468.2386.

2,6-*O*-Dibenzyl-4-*C*-napthyl-*myo*-inositol (151g)



2,6-*O*-Dibenzyl-4-*C*-naphthyl-*myo*-inositol 3,5-*O*-benzylidene acetal (**176g**, 105 mg, 0.18 mmol) was taken up in CH_2Cl_2-EtOH-H_2O (5:1:1 v/v, 1.4 mL) and *p*-TsOH (7 mg, 0.04 mmol) added. After 24 h, all solvents were evaporated and the crude material purified by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 4:1 v/v) afforded the *title compound* (**151g**, 38 mg, 43 %) as a clear glass; R_f (EtOAc-hexane 7:3 v/v) 0.26; δ_H (400 MHz, $CDCl_3$) 7.87-7.79 (4H, m), 7.61 (1H, d, *J* 8.4), 7.48-7.46 (2H, m), 7.36-7.28 (8H, m), 7.19-7.17 (2H, m) (17 × Ar ***H***), 4.67 (1H, d, *J* 11.6), 4.63 (1H, d, *J* 11.6), 4.59 (1H, d, *J* 11.6), 4.52 (1H, d, *J* 11.6) (4 × OCH***H***Ph), 4.30-4.27 (1H, bs, Ins ***H***), 4.15-4.13 (1H, m, Ins ***H***), 4.00-3.96 (2H, m, 2 × Ins ***H***), 3.67-3.65 (1H, bs, Ins ***H***), 3.42 (1H, d, *J* 13.6), 3.37 (1H, d, *J* 13.6) (2 × 4-CH***H***Naphthyl) ppm; δ_C (125 MHz, $CDCl_3$) 137.37, 136.47, 134.20, 133.39, 132.33 (5 × Ar ***C***), 129.93, 129.69, 128.69 (2C), 128.61 (2C), 128.38, 128.29, 128.14 (2C), 127.95 (2C), 127.82, 127.55, 127.12, 125.61, 125.25 (17 × Ar ***C***H), 80.17 (2 × Ins ***C***H), 75.48 (Ins ***C***), 74.00 (Ins ***C***H), 73.45, 71.48 (2 × O***C***H_2Ph), 71.14, 69.63 (2 × Ins ***C***H), 41.37 (4-***C***H_2Ph) ppm; HRMS (ESI^+) found $[M+Na]^+$ 523.2110 (100), $C_{31}H_{32}O_6Na$ requires 523.2097.

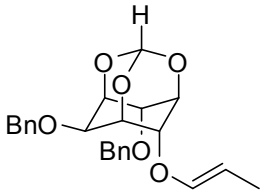
1,3,4,5-*O*-Tetraacetyl-2,6-*O*-dibenzyl-4-*C*-methyl-*myo*-inositol (152)



2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol (**151a**, 60 mg, 0.16 mmol) and DMAP (2 mg, 0.02 mmol) were evaporated from pyridine (3 × 1 mL) and taken up in CH_2Cl_2 (1 mL). Acetic anhydride (127 μL, 1.28 mmol) and triethylamine (267 μL, 1.92 mmol) were added and the reaction mixture stirred for 2 h. The reaction was quenched by drop-wise addition of H_2O (0.5 mL) and stirred for a further 30 min before the product was taken up in CH_2Cl_2, washed with H_2O, then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 7:10 v/v) afforded the *title compound* (**152**, 45 mg, 52 %) as a colourless oil; R_f (EtOAc) 0.9; δ_H (400 MHz, $CDCl_3$) 7.41-7.25 (10H, m, 10 × Ar ***H***), 6.14 (1H, d, *J* 3.6 Ins 3-***H***), 6.03 (1H, d, *J* 9.6, Ins 5-***H***), 5.09 (1H, dd, *J* 11.2, 2.8, Ins 1-***H***), 4.72-4.61 (4H, m, 2 × OC***H₂***Ph), 4.18 (1H, t, *J* 3.2, Ins 2-***H***), 3.99 (1H, t, *J* 10.0, Ins 6-***H***), 2.08 (3H, s), 2.02 (3H, s), 1.96 (3H, s), 1.91 (3H, s) (4 × OCOC***H₃***), 1.59 (3H, s, Ins 4-C***H₃***) ppm; δ_C (100 MHz, $CDCl_3$) 170.31, 169.70, 169.58, 169.35 (4 ×

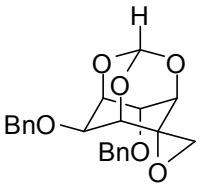
O***C***OCH$_3$), 138.24, 137.99 (2 × Ar ***C***), 128.39 (2C), 128.33 (2C), 127.83, 127.64 (2C), 127.56, 127.25 (2C) (10 × Ar ***C***H), 84.52 (Ins 4-***C***), 76.77, 76.21 (2 × Ins ***C***H), 75.55, 74.97 (2 × O***C***H$_2$Ph), 72.86, 72.48, 70.32 (3 × Ins ***C***H), 22.33, 20.78, 20.74, 20.71 (4 × OCO***C***H$_3$), 17.42 (Ins 4-***C***H$_3$) ppm.

2,6-*O*-Dibenzyl-4-*O*-(prop-1-enyl)-*myo*-inositol 1,3,5-orthoformate (159)



2,6-*O*-Dibenzyl-4-*O*-allyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (3.85 g, 9.39 mmol) was evaporated from MeCN (3 × 5 mL), dissolved in DMSO (10 mL) and potassium *t*-butoxide (2.11 g, 18.75 mmol) added. The solution was stirred at 100 °C for 3 h. The reaction was cooled, diluted with H_2O (5 mL) and extracted with EtOAc. The organic layer was washed with H_2O (× 4), then brine, dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The *title compound* (3.85 g, 100 %) was isolated as a yellow oil and used without further purification; R_f (EtOAc-hexane 1:1 v/v) 0.65, δ_H (400 MHz, $CDCl_3$) 7.43-7.26 (10H, m, 10 × Ar ***H***), 6.00 (1H, dq *J* 6.0, 1.6, OCHC***H***CH$_3$), 5.57 (1H, d, *J* 1.0, O$_3$C***H***), 4.73 (1H, d, *J* 12.3, OCH***H***Ph), 4.70 (1H, d, *J* 12.2, OCH***H***Ph), 4.64 (1H, d, *J* 11.8, OCH***H***Ph), 4.55-4.43 [3H, m, OC***H***CHCH$_3$ + (2 × Ins ***H***)], 4.48 (1H, d, *J* 12.1, OCH***H***Ph), 4.37-4.29 (3H, m, 3 × Ins ***H***). 4.03 (1H, q, *J* 1.4, Ins 2-***H***), 1.38 (3H, dd, *J* 6.8, 1.3, OCHCHC***H$_3$***) ppm; MS (ESI$^+$) *m/z* (%) found $[M+H]^+$ 411 (62), $[M+Na]^+$ 433 (67); HRMS (ESI$^+$) found 411.1812, $C_{24}H_{27}O_6$ requires 411.1808.

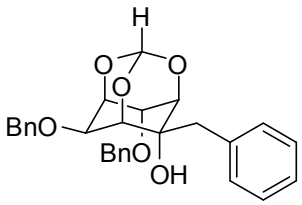
2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthoformate (160)



To a clear solution of trimethylsulfoxonium iodide (759 mg, 3.45 mmol) in DMSO (6 mL) was added NaH (60 % dispersion in mineral oil, 150 mg, 3.76 mmol) and the mixture stirred for 40 min. 2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-*O*-orthoformate (**42**, 1.15 g, 3.13 mmol) was evaporated from MeCN (3 × 5 mL), taken up in THF (6 mL) and added to the reaction mixture, which was stirred at rt for 1 h. The reaction was quenched by drop-wise addition of H_2O (2 mL) and the product taken up in EtOAc. The organic layer was washed with sat. $NaHCO_3$, H_2O, then brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 2:5 v/v)

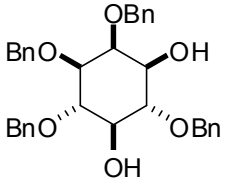
afforded the *title compound* (**160**, 440 mg, 37 %) as a clear oil; R_f (EtOAc- hexane, 1:1 v/v) 0.47; δ_H (400 MHz, $CDCl_3$) 7.42-7.29 (10H, m, 10 × Ar ***H***), 5.67 (1H, d, *J* 1.0, O_3C***H***), 4.70 (1H, d, *J* 12.0), 4.64 (1H, d, *J* 12.0), 4.62 (1H, d, *J* 11.8) 4.46 (1H, d, *J* 11.8) (4 × OCH***H***Ph), 4.34-4.32 (1H, m, Ins 1-***H***), 4.32 (1H, t, *J* 4.4 Ins 6-***H***), 4.00 (1H, q, *J* 1.2, Ins 2-***H***), 3.84 (1H, td, *J* 3.9, 2.0, Ins 5-***H***), 3.78 (1H, q, *J* 2.0, Ins 3-***H***), 2.91 (1H, d, *J* 4.4), 2.88 (1H, d, *J* 4.4) (2 × Ins 4-CCH***H***) ppm; δ_C (100 MHz, $CDCl_3$) 137.58, 137.30 (2 × Ar ***C***), 128.57 (2C), 128.52 (2C), 128.11, 127.97 (3C), 127.76 (2C) (10 × Ar ***C***H), 103.42 (H***C***O_3), 73.62, 72.48 (2 × Ins ***C***H), 71.60 (O***C***H_2Ph), 71.58 (Ins ***C***H), 71.39 (O***C***H_2Ph), 70.19, 69.34 (2 × Ins ***C***H), 60.37 (Ins 4-C***C***HH), 54.91 (Ins ***C***) ppm; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 383.1483 (72), $C_{22}H_{23}O_6$ requires 383.1495.

2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (161)



2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthoformate (**160**, 440 mg, 1.15 mmol) was treated with phenyl lithium cuprate according to general method 1. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 3:7 v/v) afforded the *title compound* (**161**, 464 mg, 87 %) as a clear oil; R_f (EtOAc-hexane, 3:7 v/v) 0.61; δ_H (400 MHz, $CDCl_3$) 7.43-7.28 (13H, m), 7.15-7.12 (2H, m) (15 × Ar ***H***), 5.64 (1H, d, *J* 0.7, O_3C***H***) 4.75 (1H, d, *J* 12.3), 4.62 (1H, d, *J* 12.3), 4.51 (1H, d, *J* 11.5), 4.44 (1H, d, *J* 11.5) (4 × OCH***H***Ph), 4.38 (1H, t, *J* 3.9, Ins 6-***H***), 4.27 (1H, dq, *J* 3.9, 2.0, Ins 1-***H***), 4.23 (1H, bs, Ins 4-O***H***), 4.04 (1H, q, *J* 2.0, Ins 3-***H***), 3.96 (1H, q, *J* 1.5, Ins 2-***H***), 3.85 (1H, dt, *J* 3.9, 2.0, Ins 5-***H***), 3.34 (1H, d, *J* 13.6), 3.18 (1H, d, *J* 13.6) (2 × CH***H***Ph) ppm; δ_C (125 MHz, $CDCl_3$) 137.68, 135.99, 135.81 (3 × Ar ***C***), 131.05 (2C), 128.83 (2C), 128.76, 128.57 (2C), 128.12 (3C), 128.08 (3C), 128.00, 126.57 (2C) (15 × Ar ***C***H), 102.89 (H***C***O_3), 74.74, 74.28 (2 × Ins ***C***H), 73.05, 71.55 (2 × O***C***H_2Ph), 71.40 (Ins ***C***), 69.61, 69.52, 67.52 (3 × Ins ***C***H), 41.56 (C***C***H_2Ph) ppm; HRMS (CI^+) *m/z* (%) found $[M+H]^+$ 461.1951 (50), $C_{28}H_{29}O_6$ requires 461.1964.

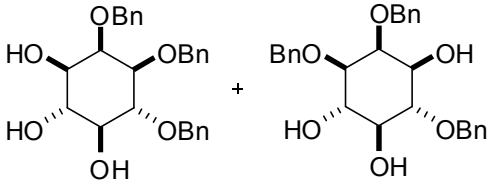
1,2,4,6-*O*-Tetrabenzyl-*myo*-inositol (164)



2,4,6-*O*-Tribenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**162**, 100 mg, 0.19 mmol) was reduced with DIBAL-H using general method 2, affording **164** (102 mg, 100 %); R_f (EtOAc-

hexane, 1:1 v/v) 0.48; δ_H (400 MHz, $CDCl_3$) 7.41-7.29 (20H, m, 20 × Ar ***H***), 5.03 (1H, d, *J* 11.6), 5.02 (1H, d, *J* 11.2), 4.93 (1H, d, *J* 11.3), 4.82 (1H, d, *J* 11.4), 4.80 (1H, d, *J* 11.2), 4.77 (1H, d, *J* 11.6), 4.71 (2H, s) (8 × OC***H₂***Ph), 4.09 (1H, t, *J* 2.5, Ins 2-***H***), 3.95 (1H, t, *J* 9.4, Ins 4-***H***), 3.74 (1H, t, *J* 9.3, Ins 6-***H***), 3.58 (1H, t, *J* 9.1, Ins 5-***H***), 3.52 (1H, bd, *J* 10.1, Ins 1-***H***), 3.48 (1H, dd, *J* 9.7, 2.4, Ins 3-***H***), 2.57 (1H, bs, Ins 5-O***H***), 2.38 (1H, bs, Ins 1-O***H***) ppm; δ_C (100 MHz, $CDCl_3$) 138.66, 138.62 (2C), 138.05 (4 × Ar ***C***), 128.50 (4C), 128.45 (2C), 128.37 (2C), 128.03 (2C), 128.00 (2C), 127.77 (4C), 127.75, 127.65, 127.60 (2C) (20 × Ar ***C***H), 81.66 (Ins 6-***C***H), 81.25 (Ins 4-***C***H), 80.87 (Ins 3-***C***H), 77.05 (Ins 2-***C***H), 75.48 (O***C***H_2Ph), 74.98 (Ins 5-***C***H), 74.98, 74.94, 72.70 (3 × O**C**H_2Ph), 72.18 (Ins 1-***C***H) ppm; MS (CI^+) *m/z* (%) $[M+H]^+$ 540 (100).

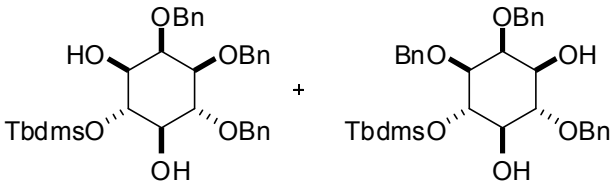
1,2,6-*O*-Tribenzyl-*myo*-inositol (165) and 2,3,6-*O*-tribenzyl-*myo*-inositol (166)



2,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**95**, 225 mg, 0.50 mmol) was reduced with DIBAL-H using general method 2. The crude residue was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (9:1 → 2:8 v/v) afforded **165** (40 mg, 18 %) and **166** (60 mg, 26 %) both as off-white solids; for **165** R_f (EtOAc-hexane, 7:3 v/v) 0.18; δ_H (500 MHz, d_6-DMSO) 7.31-7.21 (15H, m, 15 × Ar C***H***), 4.88 (1H, d, *J* 5.2, ex, Ins 5-O***H***), 4.81-4.70 [6H, m, Ins 3-O***H*** (ex) + Ins 4-O***H*** (ex) + (2 × OC***H₂***Ph)], 4.62 (1H, d, *J* 11.9), 4.54 (1H, d, *J* 11.9) (2 × OCH***H***Ph), 3.98 (1H, t, *J* 2.4, Ins 2-***H***), 3.58 (1H, t, *J* 9.5, Ins 6-***H***), 3.47 (1H, td, *J* 9.5, 4.6, ex → t, Ins 4-***H***), 3.45 (1H, dd, *J* 9.9, 2.5, Ins 1-***H***), 3.27 (1H, ddd, *J* 9.8, 4.6, 2.4, ex → dd, Ins 3-***H***), 3.16 (1H, td, *J* 9.0, 5.2, ex → t, Ins 5-***H***) ppm; δ_C (125 MHz, $CDCl_3$) 138.64 (2C), 138.10 (3 × Ar ***C***), 128.48 (4C), 128.43 (2C), 128.07 (2C), 127.81 (2C), 127.78 (2C), 127.73, 127.64 (2C) (15 × Ar ***C***H), 81.11, 80.92, 77.34 (3 × Ins ***C***H), 75.48, 74.89 (2 × O***C***H_2Ph), 74.51, 73.60 (2 × Ins ***C***H), 72.85 (O***C***H_2Ph), 72.10 (Ins ***C***H) ppm; HRMS (CI^+) *m/z* (%) found $[M+Na]^+$ 473.1947 (100), $C_{27}H_{30}O_6Na$ requires 473.1940; for **166** R_f (EtOAc-hexane, 7:3 v/v) 0.46; δ_H (400 MHz, d_6-DMSO) 7.42-7.24 (15H, m, 15 × Ar C***H***), 4.96 (1H, d, *J* 5.1, ex, Ins 1-O***H***), 4.93 (1H, d, *J* 4.9, ex, Ins 4-O***H***), 4.91 (1H, d, *J* 5.2, ex, Ins 6-O***H***), 4.83 (1H, d, *J* 11.9), 4.81 (1H, d, *J* 11.5), 4.76 (1H, d, *J* 11.5), 4.73 (1H, d, *J* 11.9), 4.64 (2H, s) (6 × OCH***H***Ph), 3.96 (1H, bs, Ins 2-***H***), 3.64 (1H, td, *J* 9.4, 4.9, ex → t, Ins 4-***H***), 3.51-3.44 (2H, m, Ins 5-***H*** + Ins 1-***H***), 3.25 (1H, dd, *J* 9.9, 2.3, Ins 3-***H***), 3.18 (1H, td, *J* 8.8, 5.2, ex → t, Ins 6-***H***) ppm; δ_C (100 MHz, d_6-DMSO) 140.23, 140.06, 139.59 (3 × Ar ***C***), 128.57 (2C), 128.45 (2C), 128.33 (2C), 128.03, 127.84 (2C), 127.67 (4C), 127.53, 127.43 (15 × Ar ***C***H), 82.45, 80.56, 79.42, 75.60 (4 × Ins ***C***H), 74.51, 74.10 (2 × O***C***H_2Ph), 73.17, 71.97 (2 × Ins

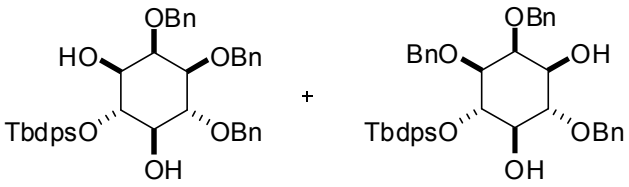
CH), 71.91 (O***C***H$_2$Ph) ppm; HRMS (CI$^+$) *m/z* (%) found [M+Na]$^+$ 473.1947 (100), $C_{27}H_{30}O_6Na$ requires 473.1940.

1,2,6-*O*-Tribenzyl-4-*O*-*tert*-butyldimethylsilyl-*myo*-inositol (169) and 2,3,6-*O*-tribenzyl-4-*O*-*tert*-butyldimethylsilyl-*myo*-inositol (170)



2,6-*O*-Dibenzyl-4-*O*-*tert*-butyldimethylsilyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**167**, 100 mg, 0.15 mmol) was reduced by DIBAL-H using general method 2. The residue (86 mg) was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (9:1 → 3:7 v/v) afforded **169** (40 mg, 40 %) and **170** (42 mg, 42 %); for **169** R_f (EtOAc-hexane, 1:1 v/v) 0.72; δ_H (400 MHz, $CDCl_3$) 7.41-7.31 (15H, m, 15 × Ar ***H***), 5.02 (1H, d, *J* 12.1, 2-OC***H***HPh), 4.99 (1H, d, *J* 11.9), 4.78 (1H, d, *J* 11.4) (2 × 6-OC***H***HPh), 4.75 (1H, d, *J* 12.1, 2-OC***H***HPh), 4.70 (2H, s, 1-OC***H$_2$***Ph), 4.06 (1H, t, *J* 2.6, Ins 2-***H***), 3.90 (1H, t, *J* 9.5, Ins 6-***H***), 3.80 (1H, t, *J* 9.1, Ins 4-***H***), 3.48 (1H, dd, *J* 9.7, 2.4, Ins 1-***H***), 3.37 (1H, t, *J* 9.0, Ins 5-***H***), 3.34 (1H, dd, *J* 9.4, 2.6, Ins 3-***H***), 2.43 (1H, bs, Ins O***H***), 2.17 (1H, bs, Ins O***H***), 0.93 (9H, s, SiC***Me$_3$***), 0.14 (3H, s), 0.13 (3H, s) (2 × Si***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 128.72 (2C), 138.7 (3 × Ar ***C***), 128.41 (2C), 128.38 (3C), 127.89 (2C), 127.70, 127.66 (2C), 127.64 (3C), 127.61 (2C) (15 × Ar ***C***H), 81.14, 80.82, 77.09, 75.47 (4 × Ins ***C***H), 75.47 (O***C***H$_2$Ph), 75.23 (Ins ***C***H), 74.66 (O***C***H$_2$Ph), 72.95 (Ins ***C***H), 72.70 (O***C***H$_2$Ph), 25.93 (SiC***Me$_3$***), 18.30 (Si***C***Me$_3$), -4.28, -4.44 (2 × Si***Me***) ppm; HRMS (ESI$^+$) *m/z* (%) found [M+H]$^+$ 565.2992 (100), $C_{33}H_{45}O_6Si$ requires 565.2985; for **170** R_f (EtOAc-hexane, 1:1 v/v) 0.63; δ_H (400 MHz, $CDCl_3$) 7.41-7.30 (15H, m, 15 × Ar ***H***), 4.94 (1H, d, *J* 11.6, 6-OC***H***HPh), 4.91 (1H, d, *J* 11.5), 4.83 (1H, d, *J* 11.2) (2 × 2-OC***H***HPh), 4.69 (1H, d, *J* 11.5, 6-OC***H***HPh), 4.66 (2H, s, 3-OC***H$_2$***Ph), 4.04 (1H, t, *J* 9.1, Ins 4-***H***), 4.02 (1H, t, *J* 2.8, Ins 2-***H***), 3.68 (1H, t, *J* 9.3, Ins 6-***H***), 3.53 (1H, ddd, *J* 9.2, 6.4, 2.6, ex → dd, Ins 1-***H***), 3.45 (1H, td, *J* 8.9, 2.1, ex → t, Ins 5-***H***), 3.27 (1H, dd, *J* 9.5, 2.2, Ins 3-***H***), 2.44 (1H, d, *J* 2.2, Ins 5-O***H***), 2.33 (1H, d, *J* 6.6, Ins 1-O***H***), 0.92 (9H, s, SiC***Me$_3$***), 0.17 (3H, s), 0.09 (3H, s) (2 × Si***Me***) ppm; δ_C (125 MHz, $CDCl_3$) 138.79, 138.70, 137.99 (3 × Ar ***C***), 128.47 (2C), 128.35 (3C), 127.98 (2C), 127.73, 127.62 (7C) (15 × Ar ***C***H), 81.77, 80.91, 77.11, 76.09 (4 × Ins ***C***H), 74.96, 74.79 (2 × O***C***H$_2$Ph), 74.07 (Ins ***C***H), 72.73 (O***C***H$_2$Ph), 72.32 (Ins ***C***H), 25.99 (SiC***Me$_3$***), 18.31 (Si***C***Me$_3$), -4.06, -4.55 (2 × Si***Me***) ppm; HRMS (ESI$^+$) *m/z* (%) found [M+H]$^+$ 565.2972 (100), $C_{33}H_{45}O_6Si$ requires 565.2985.

1,2,6-*O*-Tribenzyl-4-*O*-*tert*-butyldiphenylsilyl-*myo*-inositol (171) and 2,3,6-*O*-tribenzyl-4-*O*-*tert*-butyldiphenylsilyl-*myo*-inositol (172)

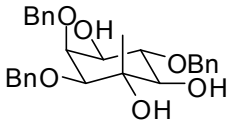


2,6-*O*-Dibenzyl-4-*O*-*tert*-butyldiphenylsilyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**168**, 460 mg, 1.07 mmol) was reduced with DIBAL-H using general method 2. The residue (430 mg) was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (9:1 → 7:3 v/v) afforded **171** (193 mg, 42 %) and **172** (153 mg, 33 %); for **171** R_f (EtOAc-hexane, 3:7 v/v) 0.62; δ_H (400 MHz, $CDCl_3$) 7.75-7.72 (4H, m), 7.44-7.26 (19H, m), 7.07-7.05 (2H, m) (25 × Ar ***H***), 4.88 (2H, d, *J* 11.3, 2-OC***H***HPh + 6-OC***H***HPh), 4.75 (1H, d, *J* 10.9, 6-OC***H***HPh), 4.69 (1H, d, *J* 11.9), 4.66 (1H, d, *J* 11.8) (2 × 1-OC***H***HPh), 4.49 (1H, d, *J* 11.8, 2-OC***H***HPh), 3.90 (1H, t, *J* 2.6, Ins 2-***H***), 3.89 (1H, t, *J* 8.9, Ins 4-***H***), 3.77 (1H, t, *J* 9.3, Ins 6-***H***), 3.59 (1H, td, *J* 8.9, 2.3, Ins 5-***H***), 3.46 (1H, dd, *J* 9.5, 2.6, Ins 1-***H***), 3.45 (1H, td, *J* 8.8, 2.7, Ins 3-***H***), 2.44 (1H, d, *J* 2.4, Ins 5-O***H***), 1.68 (1H, d, *J* 8.4, Ins 3-O***H***), 1.21 (9H, s, SiC***Me₃***) ppm; δ_C (100 MHz, $CDCl_3$) 138.81, 138.61, 138.13 (3 × Ar ***C***), 135.85 (2C), 135.82 (2C) (4 × Ar ***C***H), 133.85 (2 × Ar ***C***), 129.66, 129.61, 128.40 (2C), 128.32 (2C), 128.21 (2C), 127.86 (2C), 127.71 (2C), 127.69, 127.61 (2C), 127.59 (2C), 125.53, 127.31, 127.12 (2C) (21 × Ar ***C***H), 81.12, 80.66, 77.59, 76.47, 75.53 (5 × Ins ***C***H), 75.51, 74.39 (2 × O***C***H_2Ph), 72.82 (Ins ***C***H), 72.73 (O***C***H_2Ph), 27.09 (SiC***Me₃***), 19.71 (Si***C***Me_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 711.3106 (100), $C_{43}H_{48}O_6SiNa$ requires 711.3118; for **172** R_f (EtOAc-hexane, 3:7 v/v) 0.34; δ_H (400 MHz, $CDCl_3$) 7.76-7.68 (4H, m), 7.45-7.18 (17H, m), 7.14-7.12 (2H, m), 6.96-6.94 (2H, m) (25 × Ar ***H***), 4.88 (1H, d, *J* 11.2), 4.71 (1H, d, *J* 11.2) (2 × 6-OC***H***HPh), 4.59 (1H, d, *J* 11.5), 4.53 (1H, d, *J* 11.4) (2 × 2-OC***H***HPh), 4.41 (1H, d, *J* 11.3, 3-OC***H***HPh), 4.27 (1H, t, *J* 8.7, Ins 4-***H***), 4.19 (1H, d, *J* 11.3, 3-OC***H***HPh), 4.04 (1H, t, *J* 2.1, Ins 2-***H***), 3.70 (1H, td, *J* 8.0, 2.7, Ins 5-***H***), 3.67-3.60 (2H, m, Ins 1-***H*** + Ins 6-***H***), 3.41 (1H, dd, *J* 9.0, 2.1, Ins 3-***H***), 2.41 (1H, d, *J* 3.0, Ins 5-O***H***), 2.35 (1H, d, *J* 4.3, Ins 1-O***H***), 1.04 (9H, s, SiC***Me₃***) ppm; δ_C (100 MHz, $CDCl_3$) 138.69, 138.65, 137.62 (3 × Ar ***C***), 135.94 (2C), 135.87 (2C) (4 × Ar ***C***H), 133.88, 133.77 (2 × Ar ***C***), 129.48, 129.44,. 128.42 (2C), 128.18 (2C), 128.02 (2C), 127.93 (2C), 127.70 (2C), 127.56 (2C), 127.45 (2C), 127.36 (5C) (21 × Ar ***C***H), 81.60, 80.95, 76.29, 75.83, 75.03 (5 × Ins ***C***H), 74.88, 74.11 (2 × O***C***H_2Ph), 72.23 (Ins ***C***H), 71.77 (O***C***H_2Ph), 27.06 (SiC***Me₃***), 19.65 (Si***C***Me_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 711.3102 (100), $C_{43}H_{48}O_6SiNa$ requires 711.3118.

Stirring **171** or **172** (155 mg, 0.225 mmol) with TBAF (1 M solution in THF, 1.125 mmol) in THF (1 mL) for 16 h, followed by purification by chromatography on flash silica (elution with

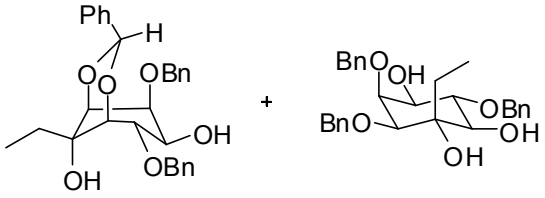
EtOAc-hexane 1:9 → 7:3 v/v) isolated **165** from **171** (70 mg, 69 %) and **166** from **172** (93 mg, 92 %), as characterized previously.

2,3,6-*O*-Tribenzyl-4-*C*-methyl-*myo*-inositol (175a)



2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**115**, 100 mg, 0.21 mmol) was treated with DIBAL-H according to general method 3. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 2:5 v/v) afforded the *title compound* (**175a**, 55 mg, 55 %) as a clear glass; R_f (EtOAc-hexane, 3:7 v/v) 0.09; δ_H (500 MHz, CD_3OD) 7.44-7.21 (15H, m, 15 × Ar ***H***), 4.84-4.81 (3H, m, 6-OC***H*****$_2$**Ph, 2-OCH***H***Ph), 4.73 (1H, d, *J* 11.7, 3-OCH***H***Ph), 4.72 (1H, d, *J* 11.4, 2-OCH***H***Ph), 4.66 (1H, d, *J* 11.7, 3-OCH***H***Ph), 3.99 (1H, t, *J* 2.8, Ins 2-***H***), 3.86 (1H, bs, Ins O***H***), 3.74 (1H, bs, Ins O***H***), 3.55-3.50 (2H, m, Ins 1-***H*** + Ins 6-***H***), 3.39 (1H, d, *J* 8.9, Ins 5-***H***), 3.37 (1H, d, *J* 2.8, Ins 3-***H***), 2.79 (1H, bs, Ins O***H***), 1.41 (3H, s, Ins 4-C***H*****$_3$**) ppm; δ_C (125 MHz, CD_3OD) 140.50, 140.40, 140.22 (3 × Ar ***C***), 129.29 (2C), 129.45 (2C), 128.14 (2C), 129.10 (2C), 128.93 (2C), 128.83 (2C), 128.53, 128.46, 128.32 (15 × Ar ***C***H), 84.04, 82.55, 79.23, 78.89 (4 × Ins ***C***H), 78.24 (Ins ***C***), 76.09, 75.88, 74.35 (3 × O***C***H_2Ph), 73.31 (Ins ***C***H), 17.70 (Ins 4-***C***H_3) ppm; HRMS (CI^+) *m/z* (%) found $[M+NH_4]^+$ 482.2545 (100), $C_{28}H_{36}NO_6$ requires 482.2542.

2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol 3,5-*O*-benzylidene acetal (176b) and 2,3,6-*O*-tribenzyl-4-*C*-ethyl-*myo*-inositol (175b)



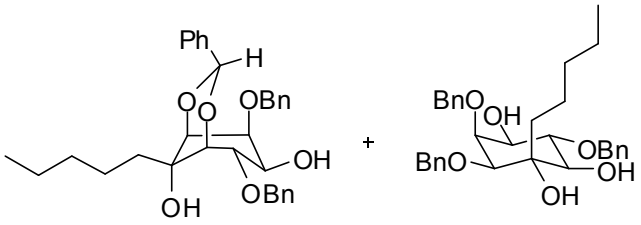
2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**136**, 220 mg, 0.46 mmol) was treated with DIBAL-H for 12 h, according to general method 3. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 1:1 v/v) afforded the benzylidene acetal (**176b**, 42 mg, 19 %) and tribenzyl (**175b**, 72 mg, 32 %).

Treatment of 2,6-*O*-dibenzyl-4-*C*-ethyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**136**, 350 mg, 0.74 mmol) with. DIBAL-H according to general method 3 for 48 h afforded only the tribenzyl (**175b**, 216 mg, 61 %) after purification by chromatography on flash silica as detailed above.

For 2,6-*O*-dibenzyl-4-*C*-ethyl-*myo*-inositol 3,5-*O*-benzylidene acetal, **176b**: R_f (EtOAc-hexane, 3:7 v/v) 0.23; δ_H (500 MHz, d_6-DMSO) 7.40-7.25 (15H, m, 15 × Ar ***H***), 5.98 (1H, s, PhC***H***O_2), 4.93 (1H, s, ex, Ins 4-O***H***), 4.74 (1H, d, *J* 12.1, 2-OCH***H***Ph), 4.70 (2H, s, 6-OC***H***$_2$Ph), 4.67 (1H, d, *J* 7.4, ex, Ins 1-O***H***), 4.64 (1H, d, *J* 12.1, 2-OCH***H***Ph), 4.46 (1H, q, *J* 7.7, ex → t, Ins 1-***H***), 4.20 (1H, dd, *J* 6.6, 1.9, Ins 3-***H***), 4.13 (1H, dd, *J* 8.2, 6.8, Ins 2-***H***), 3.94 (1H, d, *J* 6.3, Ins 6-***H***), 3.83 (1H, d, *J* 1.7, Ins 5-***H***), 2.01 (1H, dq, *J* 14.3, 6.9), 1.90 (1H, dq, *J* 14.3, 7.7) (2 × C***H***HCH_3), 0.90 (3H, t, *J* 7.4, CH_2C***H***$_3$) ppm; δ_C (100 MHz, d_6-DMSO) 139.20 (2C), 139.16 (3 × Ar ***C***), 129.12 , 128.60 (2C), 128.57 (2C), 128.50 (2C), 128.09 (2C), 127.98 (2C), 127.79, 127.78, 126.73 (2C) (15 × Ar ***C***H), 92.57 (Ph***C***HO_2), 84.95, 77.48, 72.92, 72.46 (4 × Ins ***C***H), 72.00, 71.60 (2 × O***C***H_2Ph), 69.36 (Ins ***C***H), 69.02 (Ins ***C***), 28.75 (***C***H_2CH_3), 7.06 (CH_2***C***H_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 499.2093 (100), $C_{29}H_{32}O_6Na$ requires 499.2097.

For 2,3,6-*O*-tribenzyl-4-*C*-ethyl-*myo*-inositol, **175b**: R_f (EtOAc-hexane, 3:7 v/v) 0.32; δ_H (400 MHz, $CDCl_3$) 7.42-7.27 (15H, m, 15 × Ar ***H***), 4.91 (1H, d, *J* 10.8), 4.77 (1H, d, *J* 11.2), 4.70 (1H, d, *J* 11.7), 4.69 (1H, d, *J* 11.2), 4.67 (1H, d, *J* 10.8), 4.63 (1H, d, *J* 11.7) (6 × OCH***H***Ph), 4.37 (1H, m, Ins ***H***), 4.16 (1H, t, *J* 3.2, Ins 2-***H***), 4.16-4.13 (1H, m), 3.92 (1H, m), 3.74 (1H, m) (3 × Ins ***H***), 2.11 (1H, dq, *J* 13.7, 6.8), 1.77 (1H, dq, *J* 13.7, 7.3) (2 × CH***H***CH_3), 1.03 (3H, t, *J* 7.3, CH_2C***H***$_3$) ppm; δ_C (100 MHz, $CDCl_3$) 137.89, 137.74, 136.80 (3 × Ar ***C***), 128.70 (2C), 128.56 (4C), 128.36, 127.99, 127.93 (5C), 127.73 (2C) (15 × Ar ***C***H), 83.94, 80.51 (2 × Ins ***C***H), 76.63 (O***C***H_2Ph), 75.90 (Ins ***C***), 73.56 (Ins ***C***H), 73.47, 71.06 (2 × O***C***H_2Ph), 70.70, 69.48 (2 × Ins ***C***H), 27.77 (***C***H_2CH_3), 6.42 (CH_2***C***H_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 501.2251 (100), $C_{29}H_{34}O_6Na$ requires 501.2253.

2,6-*O*-Dibenzyl-4-*C*-pentyl-*myo*-inositol 3,5-*O*-benzylidene acetal (176c) and 2,3,6-*O*-tribenzyl-4-*C*-pentyl-*myo*-inositol (175c)



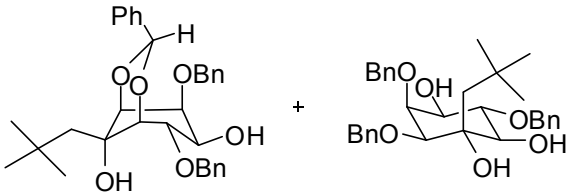
2,6-*O*-Dibenzyl-4-*C*-pentyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**133**, 510 mg, 0.99 mmol) was treated with DIBAL-H according to general method 3. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 3:2 v/v) afforded the benzylidene acetal (**176c**, 68 mg, 13 %) and tribenzyl (**175c**, 276 mg, 54 %).

For 2,6-*O*-dibenzyl-4-*C*-pentyl-*myo*-inositol 3,5-*O*-benzylidene acetal, **176c**: R_f (EtOAc-hexane, 3:7 v/v) 0.39; δ_H (500 MHz, d_6-DMSO) 7.40-7.25 (15H, m, 15 × Ar ***H***), 5.99 (1H, s, PhC***H***O_2), 4.94 (1H, s, ex, Ins 4-O***H***), 4.73 (1H, d, *J* 11.8, 2-OCH***H***Ph), 4.70 (2H, s, 6-OC***H***$_2$Ph),

4.66 (1H, d, *J* 7.4, ex, Ins 1-O***H***), 4.64 (1H, d, *J* 12.1, 2-OCH***H***Ph), 4.45 (1H, q, *J* 7.3, ex → t, Ins 1-***H***), 4.20 (1H, dd, *J* 6.6, 1.7, Ins 3-***H***), 4.13 (1H, t, *J* 6.6, Ins 2-***H***), 3.93 (1H, d, *J* 6.3, Ins 6-***H***), 3.82 (1H, d, *J* 1.4, Ins 5-***H***), 2.01-1.95 (1H, m), 1.92-1.87 (1H, m) [2 × C***H***H$(CH_2)_3CH_3$], 1.43-1.39 [2H, m, $CH_2C\boldsymbol{H_2}(CH_2)_2CH_3$], 1.33-1.25 [4H, m, 2 × $(CH_2)_2(C\boldsymbol{H_2})_2CH_3$], 0.87 [3H, t, *J* 6.9, $(CH_2)_4C\boldsymbol{H_3}$] ppm; δ_C (100 MHz, d_6-DMSO) 139.24, 139.22, 139.16 (3 × Ar ***C***), 129.12 , 128.59 (2C), 128.55 (2C), 128.52 (2C), 128.07 (2C), 127.98 (2C), 127.78, 127.74, 126.71 (2C) (15 × Ar ***C***H), 92.59 (Ph***C***HO_2), 84.99, 77.75, 72.92, 72.85 (4 × Ins ***C***H), 72.00, 71.59 (2 × O***C***H_2Ph), 69.33 (Ins ***C***H), 68.99 (Ins ***C***), 36.30, 32.42, 22.67, 21.77 (4 × ***C***H_2), 14.43 (***C***H_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 541.2556 (100), $C_{32}H_{38}O_6Na$ requires 541.2566.

For 2,3,6-*O*-tribenzyl-4-*C*-pentyl-*myo*-inositol, **175c**: R_f (EtOAc-hexane, 3:7 v/v) 0.26; δ_H (400 MHz, $CDCl_3$) 7.42-7.26 (15H, m, 15 × Ar ***H***), 4.91 (1H, d, *J* 10.8), 4.77 (1H, d, *J* 11.7), 4.72-4.61 (4H, m) (6 × OCH***H***Ph), 4.39-4.36 (1H, m), 4.18-4.13 (2H, m), 3.93-3.92 (1H, m), 3.73-3.72 (1H, m) (5 × Ins ***H***), 2.02 (1H, td, *J* 13.2, 4.9), 1.75-1.67 (1H, m) [2 × CH***H***$(CH_2)_3CH_3$], 1.55-1.46 [2H, m, $CH_2C\boldsymbol{H_2}(CH_2)_2CH_3$], 1.39-1.29 [4H, m, 2 × $(CH_2)_2(C\boldsymbol{H_2})_2CH_3$], 0.93 [3H, t, *J* 6.8 $(CH_2)_4C\boldsymbol{H_3}$] ppm; δ_C (100 MHz, $CDCl_3$) 137.90 (2C), 137.75 (3 × Ar ***C***), 128.69 (2C), 128.54 (4C), 128.34, 127.97 (4C), 127.90 (2C), 127.73 (2C) (15 × Ar ***C***H), 83.97, 80.58, 76.38 (3 × Ins ***C***H), 75.89 (2 × O***C***H_2Ph), 73.62 (Ins ***C***H), 73.46 (O***C***H_2Ph), 71.17 (Ins ***C***), 69.48 (Ins ***C***H), 35.22, 32.47, 22.78, 21.64 (4 × ***C***H_2), 14.14 (***C***H_3) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 543.2723 (100), $C_{32}H_{40}O_6Na$ requires 543.2723.

2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol 3,5-*O*-benzylidene acetal (176e) and 2,3,6-*O*-tribenzyl-4-*C*-neopentyl-*myo*-inositol (175e)



2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**144**, 290 mg, 0.51 mmol) was treated with DIBAL-H according to general method 3. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 3:2 v/v) afforded the benzylidene acetal (**176e**, 170 mg, 58 %) and tribenzyl (**175e**, 36 mg, 12 %).

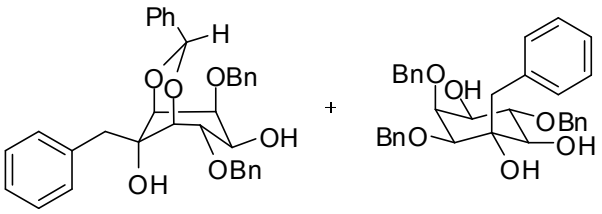
For 2,6-*O*-dibenzyl-4-*C*-neopentyl-*myo*-inositol 3,5-*O*-benzylidene acetal, **176e**: R_f (EtOAc-hexane, 3:7 v/v) 0.47; δ_H (400 MHz, d_6-DMSO) 7.42-7.27 (15H, m, 15 × Ar ***H***), 6.02 (1H, s, PhC***H***O_2), 4.73 (1H, s, ex, Ins 4-O***H***), 4.73 (1H, d, *J* 12.2, OCH***H***Ph), 4.70 (2H, s, OC***H***$_2$Ph), 4.69 (1H, d, *J* 7.3, ex, Ins 1-O***H***), 4.66 (1H, d, *J* 11.7, OCH***H***Ph), 4.44 (1H, q, *J* 7.3, ex → t, Ins 1-***H***), 4.32 (1H, dd, *J* 6.4, 1.5, Ins 3-***H***), 4.17 (1H, dd, *J* 8.3, 6.4, Ins 2-***H***), 3.93 (1H, s, Ins 5-***H***), 3.92

(1H, d, *J* 5.4, Ins 6-***H***), 2.03 (1H, d, *J* 15.2), 1.95 (1H, d, *J* 14.7) [2 × C***H***HC$(CH_3)_3$], 1.05 [9H, s, $CH_2C(C\boldsymbol{H_3})_3$] ppm; δ_C (100 MHz, d_6-DMSO) 139.36, 139.19 (2C) (3 × Ar ***C***), 129.13, 128.62 (2C), 128.55 (4C), 128.04 (2C), 127.96 (2C), 127.79 (2C), 126.83 (2C) (15 × Ar ***C***H), 92.72 (Ph***C***HO_2), 85.43, 77.97, 73.68, 72.74 (4 × Ins ***C***H), 71.99, 71.59 (2 × O***C***H_2Ph), 70.43 (Ins ***C***), 69.07 (Ins ***C***H), 47.92 [***C***$H_2C(CH_3)_3$] 32.15 [$CH_2C(\boldsymbol{C}H_3)_3$], 31.24 [$CH_2\boldsymbol{C}(CH_3)_3$] ppm; HRMS ($ESI^+$) *m/z* (%) found $[M+Na]^+$ 541.2563 (82), $C_{32}H_{38}O_6Na$ requires 541.2566.

For 2,3,6-*O*-tribenzyl-4-*C*-neopentyl-*myo*-inositol, **175e**: R_f (EtOAc-hexane, 3:7 v/v) 0.29; δ_H (400 MHz, $CDCl_3$) 7.42-7.27 (15H, m, 15 × Ar ***H***), 4.94 (1H, d, *J* 10.8), 4.77 (1H, d, *J* 11.2), 4.70 (1H, d, *J* 11.2), 4.67 (2H, d, *J* 10.3), 4.62 (1H, d, *J* 11.7) (6 × OCH***H***Ph), 4.37-4.35 (1H, m), 4.17-4.14 (2H, m), 3.94-3.93 (2H, m) (5 × Ins ***H***), 2.14 (1H, d, *J* 15.1), 1.55 (1H, d, *J* 14.7) [2 × CH***H***$C(CH_3)_3$], 1.10 [9H, s, $CH_2C(C\boldsymbol{H_3})_3$] ppm; δ_C (100 MHz, $CDCl_3$) 137.91, 137.71, 136.79 (3 × Ar ***C***), 128.71 (2C), 128.55 (4C), 128.37, 128.06 (2C), 128.01, 127.93 (3C), 127.72 (2C) (15 × Ar ***C***H), 86.11, 81.07 (2 × Ins ***C***H), 76.72, 73.48 (2 × O***C***H_2Ph), 73.33, 71.35 (2 × Ins ***C***H), 70.94 (O***C***H_2Ph), 68.98 (Ins ***C***H), 46.15 [***C***$H_2C(CH_3)_3$], 31.95 [$CH_2C(\boldsymbol{C}H_3)_3$], 31.46 [$CH_2\boldsymbol{C}(CH_3)_3$] ppm; HRMS ($ESI^+$) *m/z* (%) found $[M+Na]^+$ 543.2726 (100), $C_{32}H_{40}O_6Na$ requires 543.2723.

* No quaternary carbon was visible in the ^{13}C-NMR due to the fluxional nature of the compound.

2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 3,5-*O*-benzylidene acetal (176f) and 2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol (175f)



2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**125**, 294 mg, 0.55 mmol) was treated with DIBAL-H according to general method 3. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:1 v/v) afforded the benzylidene acetal (**176f**, 83 mg, 28 %) and tribenzyl (**175f**, 115 mg, 39 %).

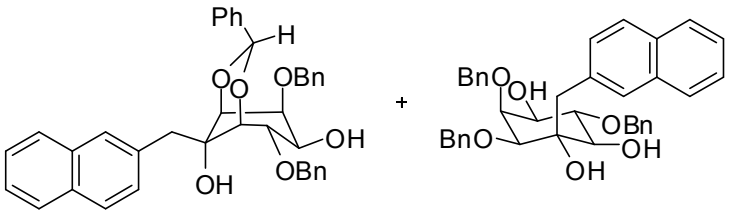
For 2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol 3,5-*O*-benzylidene acetal, **176f**: R_f (EtOAc-hexane, 3:7 v/v) 0.38; δ_H (400 MHz, d_6-DMSO) 7.50-7.44 (2H, m), 7.43-7.22 (18H, m) (20 × Ar ***H***), 6.09 (1H, s, PhC***H***O_2), 5.23 (1H, s, ex, Ins 4-O***H***), 4.72 (1H, d, *J* 7.3, ex, Ins 1-O***H***), 4.69 (1H, d, *J* 11.7), 4.65 (1H, d, *J* 11.7), 4.64 (1H, d, *J* 12.2), 4.60 (1H, d, *J* 12.2) (4 × OCH***H***Ph), 4.43 (1H, q, *J* 7.3, ex → t, Ins 1-***H***), 4.06-3.97 (2H, m, Ins 2-***H*** + Ins 3-***H***), 3.98 (1H, d, *J* 6.4, Ins 6-***H***), 3.80 (1H, s, Ins 5-***H***), 3.41 (1H, d, *J* 13.2), 3.14 (1H, d, *J* 13.7) (2 × 4-CCH***H***Ph) ppm; δ_C (100 MHz, d_6-DMSO) 139.23, 139.12 (2C), 137.41 (4 × Ar ***C***), 131.30 (2C), 128.67 (2C), 128.60 (2C), 128.52

(2C), 128.23 (2C), 128.14 (3C), 127.90 (3C), 127.79, 127.76, 126.84 (2C) (20 × Ar ***C***H), 92.96 (Ph***C***HO$_2$), 85.07, 77.72, 72.92, 72.14 (4 × Ins ***C***H), 72.12, 71.68 (2 × O***C***H$_2$Ph), 69.52 (Ins ***C***), 69.40 (Ins ***C***H), 41.94 (4-C***C***H$_2$Ph) ppm; HRMS (ESI$^+$) *m/z* (%) found [M+H]$^+$ 539.2434 (26), $C_{34}H_{35}O_6$ requires 539.2434, [M+Na]$^+$ 561.2250 (70).

For 2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol, **175f**: R_f (EtOAc-hexane, 3:7 v/v) 0.22; δ_H (400 MHz, d$_6$-DMSO) 7.44-7.41 (4H, m), 7.36-7.23 (12H, m), 7.12-7.10 (4H, m) (20 × Ar ***H***), 4.87 (1H, d, *J* 5.9, ex, Ins O***H***), 4.82 (2H, d, *J* 11.7), 4.75 (2H, d, *J* 11.7), 4.73-4.62 (1H, m), 4.45 (1H, d, *J* 11.7) (6 × OCH***H***Ph), 4.02 (1H, t, *J* 2.9, Ins 2-***H***), 3.97 (1H, s, ex, Ins O***H***), 3.76-3.65 (2H, m), 3.52-3.50 (1H, m) (3 × Ins ***H***), 3.41 (1H, s, ex, Ins O***H***), 3.36 (1H, d, *J* 9.6, Ins ***H***), 3.20 (1H, d, *J* 13.7, 4-CCH***H***Ph), 3.08 (1H, d, *J* 13.7, 4-CCH***H***Ph) ppm; HRMS (ESI$^+$) *m/z* (%) found [M+Na]$^+$ 563.2404 (100), $C_{34}H_{36}O_6Na$ requires 563.2410.

* ^{13}C-NMR too fluxional to interpret

2,6-*O*-Dibenzyl-4-*C*-(2-naphthyl)methyl-*myo*-inositol 3,5-*O*-benzylidene acetal (176g) and 2,3,6-*O*-tribenzyl-4-*C*-(2-naphthyl)methyl-*myo*-inositol (175g)



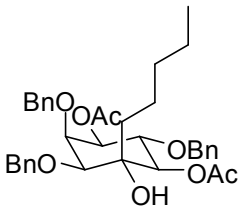
2,6-*O*-Dibenzyl-4-*C*-(2-naphthyl)methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**145**, 280 mg, 0.48 mmol) was treated with DIBAL-H according to general method 3. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 3:2 v/v) afforded the benzylidene acetal (**176g**, 118 mg, 42 %) and tribenzyl (**175g**, 82 mg, 29 %).

For 2,6-*O*-dibenzyl-4-*C*-(2-naphthyl)methyl-*myo*-inositol 3,5-*O*-benzylidene acetal, **176g**: R_f (EtOAc-hexane, 3:7 v/v) 0.42; δ_H (400 MHz, d$_6$-DMSO) 7.89-7.82 (3H, m), 7.77 (1H, s), 7.55-7.39 (8H, m), 7.36-7.23 (10H, m) (22 × Ar ***H***), 6.12 (1H, s, PhC***H***O$_2$), 5.31 (1H, s, ex, Ins 4-O***H***), 4.71 (1H, d, *J* 7.3, ex, Ins 1-O***H***), 4.68 (1H, d, *J* 11.7), 4.64 (2H, bd, *J* 9.8), 4.59 (1H, d, *J* 11.7) (4 × OCH***H***Ph), 4.42 (1H, q, *J* 6.8, ex → t, Ins 1-***H***), 4.09-4.03 (2H, m, Ins 2-***H*** + Ins 3-***H***), 3.99 (1H, d, *J* 5.9, Ins 6-***H***), 3.86 (1H, s, Ins 5-***H***), 3.58 (1H, d, *J* 13.2), 3.32 (1H, d, *J* 12.7) (2 × CH***H***Ar) ppm; δ_C (100 MHz, d$_6$-DMSO) 139.03, 138.92, 138.81, 135.10, 133.27, 132.21 (6 × Ar ***C***), 129.96, 129.47, 129.31, 128.77 (2C), 128.60 (2C), 128.55 (2C), 128.14 (2C), 127.90 (4C), 127.85 (2C), 127.46, 126.91 (2C), 126.39, 125.84 (22 × Ar ***C***H), 93.10 (Ph***C***HO$_2$), 84.78, 77.30, 72.78, 72.43 (4 × Ins ***C***H), 72.13, 71.56 (2 × O***C***H$_2$Ph), 69.62 (Ins ***C***H), 69.18 (Ins ***C***), 42.56 (***C***H$_2$Ar) ppm; HRMS (ESI$^+$) *m/z* (%) found [M+H]$^+$ 589.2597 (11), $C_{38}H_{37}O_6$ requires 589.2590, [M+Na]$^+$ 611.2384 (54).

For 2,3,6-*O*-tribenzyl-4-*C*-CH_2naphthyl-*myo*-inositol, **175g**: R_f (EtOAc-hexane, 3:7 v/v) 0.25; δ_H (400 MHz, $CDCl_3$) 7.86-7.78 (4H, m), 7.60-7.57 (1H, dd, *J* 8.3, 1.0), 7.48-7.45 (2H, m), 7.40-7.34 (10H, m), 7.28-7.27 (3H, m) (22 × Ar ***H***), 5.01 (1H, d, *J* 10.8), 4.74 (1H, d, *J* 11.7), 4.68 (1H, d, *J* 11.7), 4.66 (1H, d, *J* 10.8), 4.57 (1H, d, *J* 11.7), 4.51 (1H, d, *J* 11.2) (6 × OCH***H***Ph), 4.36-4.33 (1H, m, Ins ***H***), 4.20 (1H, t, *J* 3.4, Ins 2-***H***), 4.14-4.12 (1H, m), 3.94-3.93 (1H, m) (2 × Ins ***H***), 3.62 (1H, d, *J* 13.7, CH***H***Ar), 3.56 (1H, m, Ins ***H***), 3.12 (1H, d, *J* 12.2, CH***H***Ar) ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 613.2577 (100), $C_{38}H_{38}O_6Na$ requires 613.2566.

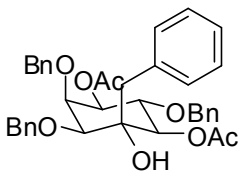
* ^{13}C-NMR spectrum too fluxional to interpret

1,5-*O*-Diacetyl-2,3,6-*O*-tribenzyl-4-*C*-pentyl-*myo*-inositol (177c)



To 2,3,6-*O*-tribenzyl-4-*C*-pentyl-*myo*-inositol (**175c**, 35 mg, 0.07 mmol) in pyridine (0.3 mL) was added DMAP (4 mg, 0.03 mmol), triethylamine (61 μL, 0.44 mmol) and acetic anhydride (35 μL, 0.37 mmol). After 18 h, H_2O (1 mL) was added and the crude material extracted into CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:1, v/v) afforded the *title compound* (**177c**, 37 mg, 98 %); R_f (EtOAc-hexane, 3:7 v/v) 0.45; δ_H (400 MHz, $CDCl_3$) 7.37-7.26 (15H, m, 15 × Ar ***H***), 5.01 (1H, d, *J* 9.6, Ins 5-***H***), 4.92 (1H, bd, *J* 6.0, Ins 1-***H***), 4.75 (1H, d, *J* 11.6), 4.70-4.63 (5H, m) (6 × OCH***H***Ph), 4.24 (1H, t, *J* 3.2, Ins 2-***H***), 3.99 (1H, t, *J* 9.6, Ins 6-***H***), 3.55 (1H, m, Ins 3-***H***), 2.06 (3H, s), 1.98 (3H, s) (2 × OCOC***H₃***), 1.71-1.66 (1H, m) [2 × CH***H***$(CH_2)_3CH_3$], 1.51-1.46 [2H, m, $CH_2CH_2(CH_2)_2CH_3$], 1.33-1.22 [4H, m, 2 × $(CH_2)_2(CH_2)_2CH_3$], 0.90 [3H, t, *J* 6.8 $(CH_2)_4CH_3$] ppm; HRMS (ESI^+) *m/z* (%) found $[M+Na]^+$ 627.2928 (100), $C_{36}H_{44}O_8Na$ requires 627.2934.

1,5-*O*-Diacetyl-2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol (177f)

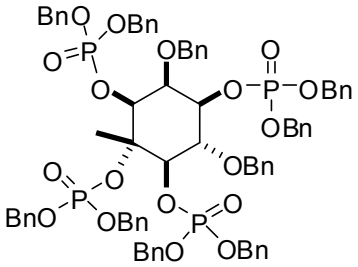


To 2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol (**175f**, 19 mg, 0.04 mmol) in pyridine (0.25 mL) was added DMAP (2 mg, 0.01 mmol), triethylamine (29 μL, 0.21 mmol) and acetic anhydride (17 μL, 0.18 mmol). After 18 h, H_2O (1 mL) was added and the crude material extracted into

CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:1, v/v) afforded the *title compound* (**177f**, 22 mg, 100 %); R_f (EtOAc-hexane, 3:7 v/v) 0.47; δ_H (400 MHz, $CDCl_3$) 7.39-7.22 (20H, m, 20 × Ar ***H***), 5.01 (1H, d, *J* 9.8, Ins 5-***H***), 4.95 (1H, bd, *J* 7.8, Ins 1-***H***), 4.84 (1H, d, *J* 12.2), 4.78-4.74 (3H, m), 4.71 (1H, d, *J* 11.7), 4.70 (1H, d, *J* 11.7) (6 × OCH***H***Ph), 4.26 (1H, t, *J* 2.9, Ins 2-***H***), 4.18 (1H, t, *J* 9.3, Ins 6-***H***), 3.64 (1H, d, *J* 2.4, Ins 3-***H***), 3.57 (1H, d, *J* 14.2), 3.25 (1H, d, *J* 14.2) (2 × 4-CCH***H***Ph), 2.72 (1H, bs, Ins 4-O***H***), 2.00 (3H, s), 1.76 (3H, s) (2 × OCOC***H₃***) ppm; δ_C (100 MHz, $CDCl_3$) 170.74, 170.19 (2 × O***C***OCH$_3$), 138.38, 138.29, 138.14, 137.55 (4 × Ar ***C***), 131.68 (2C), 128.44 (2C), 128.38 (4C), 127.87 (2C), 127.82 (3C), 127.75 (2C), 127.63 (2C), 127.33 (2C), 126.20 (20 × Ar ***C***H), 82.69, 77.76, 76.35, 75.06 (2C) (5 × Ins ***C***H), 74.99 (2C), 73.94 (3 × O***C***H$_2$Ph), 38.12 (C***C***H$_2$Ph), 20.95, 20.57 (2 × OCO***C***H$_3$) ppm; HRMS (ESI$^+$) *m/z* (%) found $[M+H]^+$ 625.2830 (7), $C_{38}H_{41}O_8$ requires 625.2801, $[M+Na]^+$ 647.2647 (100).

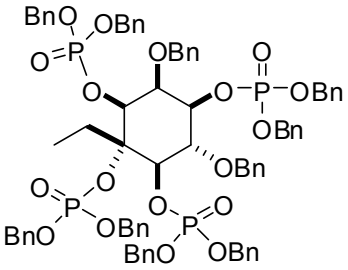
7.5 Chapter 5

1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-methyl-*myo*-inositol (179a)



2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol (**151a**, 75 mg, 0.20 mmol) was phosphitylated using *N,N*-diisopropyldibenzyl phosphoramidite and then oxidised with *m*CPBA as described in general method 7. The residue was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (3:1 → 0:1: v/v) afforded the *title compound* (**179a**, 178 mg, 63 %); R_f (EtOAc-CH_2Cl_2, 1:4 v/v) 0.52; δ_H (400 MHz, $CDCl_3$) 7.41-7.01 (50H, m, 50 × Ar ***H***), 5.12-4.67 [25H, m, (20 × Ph-CH***H***O) + (5 × Ins ***H***)], 1.31 (3H, s, Ins 4-C***H₃***) ppm; δ_P (162 MHz, $CDCl_3$) -1.46, -1.65, -1.86, -6.30 ppm; MS (MALDI-TOF) *m/z* (%) found [M+Na]+ 1438 (40).

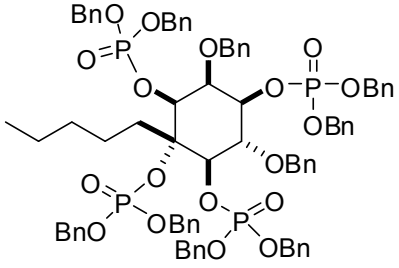
1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-ethyl-*myo*-inositol (179b)



2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol (**151b**, 72 mg, 0.19 mmol) was phosphitylated using *N,N*-diisopropyldibenzyl phosphoramidite and then oxidised with *m*CPBA as described in general method 7. The residue was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:0: v/v) afforded the *title compound* (**179b**, 82 mg, 31 %); R_f (EtOAc-hexane, 2:3 v/v) 0.05; δ_H (400 MHz, $CDCl_3$) 7.38-7.35 (2H, m), 7.27-7.23 (10H, m), 7.22-7.10 (33H, m), 7.08-7.04 (5H, m) (50 × Ar ***H***), 5.41 (1H, dd, *J* 10.5, 2.7, Ins 3-***H***), 5.12-5.04 (2H, m, Ins 5-***H*** + OCH***H***Ph), 4.99-4.72 [13H, m, (12 × OCH***H***Ph) + Ins 1-***H***], 4.65 (1H, d, *J* 11.8, OCH***H***Ph), 4.61 (1H, d, *J* 11.3, OCH***H***Ph), 4.52 (1H, t, *J* 2.8, Ins 6-***H***), 4.50 (1H, d, *J* 11.8, OCH***H***Ph), 4.33-4.30 (1H, m, Ins 2-***H***), 2.34 (2H, q, *J* 7.4, C***H₂***CH_3), 1.13 (3H, t, *J* 7.4, CH2C***H₃***) ppm; δ_C (100 MHz, $CDCl_3$) 137.90, 137.69 (2 × Ar ***C***), 136.47 (d, *J* 7.8), 136.16-135.86 (7C, m) (8 × Ar***C***CH_2OP), 128.45 (3C), 128.44 (5C), 128.34 (12C), 128.29 (5C), 128.24 (2C), 128.13 (3C), 128.12 (2C), 128.01 (2C), 127.95 (2C), 127.93 (2C), 127.87, 127.84 (2C), 127.79 (4C), 127.73 (2C), 127.68 (2C), 127.62 (50 × Ar ***C***H), 86.84 (m, Ins 4-***C***), 78.79, 77.20 (2 × Ins ***C***H), 75.13 (t, *J* 5.2), 74.14 (d, *J* 4.3) (2 × Ins ***C***H), 73.44, 72.09 (2 × O***C***H_2Ph), 71.45-71.28 (m, Ins ***C***H), 69.68 (d, *J* 6.1), 69.62 (d, *J* 6.1), 69.52 (d, *J* 6.1), 69.39 (d, *J* 5.2), 69.37 (d, *J* 5.2), 69.31 (d, *J* 5.2), 69.23 (d, *J* 5.2), 69.20 (d, *J* 5.2) (8 × PO***C***H_2Ph), 26.43 (***C***H_2CH_3), 6.50 (CH_2***C***H_3) ppm; δ_P (162 MHz, $CDCl_3$) -0.94, -1.76, -1.96, -7.07 ppm; MS (ES^+) *m/z* (%) found $[M+H]^+$ 1429 (100), $[M+Na]^+$ 1451 (57).

*Inositol peaks assigned using NOESY and COSY spectroscopy.

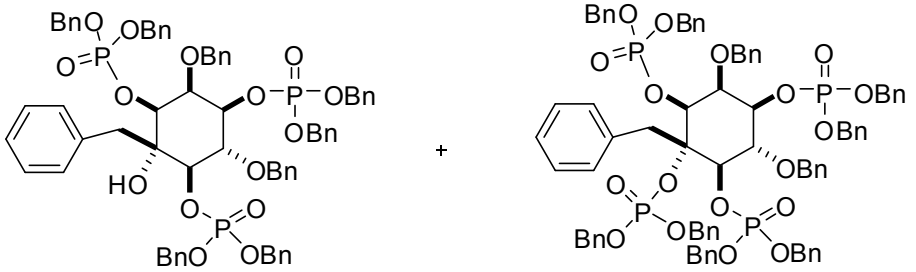
1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-pentyl-*myo*-inositol (179c)



2,6-*O*-Dibenzyl-4-*C*-pentyl-*myo*-inositol (**151c**, 71 mg, 0.17 mmol) was phosphitylated using *N,N*-diisopropyldibenzyl phosphoramidite and then oxidised with *m*CPBA as described in

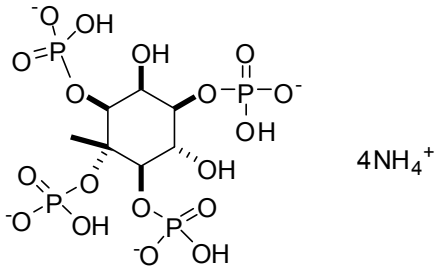
general method 7. The residue was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:4 → 1:0: v/v) afforded the *title compound* (**179c**, 69 mg, 28 %); R_f (EtOAc-hexane, 7:3 v/v) 0.52; δ_H (400 MHz, $CDCl_3$) 7.39-7.36 (2H, m), 7.32-7.11 (43H, m), 7.09-7.06 (5H, m) (50 × Ar ***H***), 5.43 (1H, dd, *J* 10.8, 2.8, Ins ***H***), 5.10-5.05 (2H, m, Ins ***H*** + OCH***H***Ph), 4.97-4.79 [13H, m, (12 × OCH***H***Ph) + Ins ***H***], 4.67 (1H, d, *J* 11.9), 4.64 (1H, d, *J* 11.5) (2 × OCH***H***Ph), 4.54-4.52 (1H, m, Ins ***H***), 4.52 (1H, d, *J* 11.9, OCH***H***Ph), 4.36-4.33 (1H, m, Ins ***H***), 2.27-2.22 (2H, m), 1.71-1.62 (2H, m), 1.14-0.85 (4H, m) (4 × C***H₂***), 0.72 (3H, t, *J* 7.0, C***H₃***) ppm; δ_C (100 MHz, $CDCl_3$) 137.89, 137.65 (2 × Ar ***C***), 136.44 (d, *J* 7.8), 136.04 (3C, d, *J* 7.8), 135.87 (3C, d, *J* 6.9), 135.82 (d, *J* 6.9) (8 × Ar ***C***CH_2OP), 128.37 (5C), 128.34 (4C), 128.29 (12C), 128.25 (5C), 128.19 (5C), 128.05 (3C), 127.95 (2C), 127.90 (2C), 127.84 (3C), 127.78 (2C), 127.74 (2C), 127.65 (2C), 127.61 (2C), 127.54 (50 × Ar ***C***H), 86.76-86.58 (m, Ins ***C***), 78.69, 77.16, 75.38 (br), 74.20 (br) (4 × Ins ***C***H), 73.40, 72.13 (2 × O***C***H_2Ph), 71.39-71.28 (m, Ins ***C***H), 69.58 (d, *J* 6.9), 69.52 (d, *J* 6.9), 69.45 (d, *J* 6.1), 69.33 (d, *J* 5.2), 69.30 (d, *J* 6.1), 69.23 (d, *J* 6.1), 69.18 (d, *J* 5.2), 69.08 (d, *J* 5.2) (8 × PO***C***H_2Ph), 33.74, 32.09, 22.51, 21.33 (4 × ***C***H_2), 14.03 (***C***H_3) ppm; δ_P (162 MHz, $CDCl_3$) -0.96, -1.73, -2.05, -7.06 ppm; MS (ES^+) *m/z* (%) found $[M+H]^+$ 1471 (100), $[M+Na]^+$ 1493 (49).

1,3,4,5-*O*-Tetrakis (dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol (179f) and 1,3,5-*O*-Tris(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol (190f)



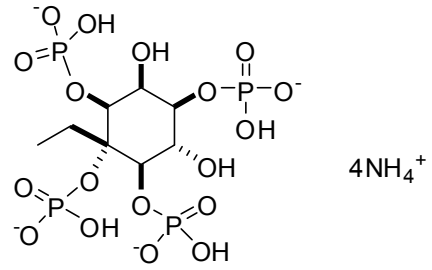
2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol (**151f**, 195 mg, 0.43 mmol) was phosphitylated using *N,N*-diisopropyldibenzyl phosphoramidite and then oxidised with *m*CPBA as described in general method 7. The residue was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 7:3: v/v) afforded an inseperable mixture of the *title compounds* (420 mg); R_f (EtOAc-hexane, 3:7 v/v) 0.21; MS (ES^+) *m/z* (%) found triphosphate $[M+H]^+$ 1231 (53), $[M+Na]^+$ 1253 (36); δ_P (162 MHz, $CDCl_3$) -0.34, -1.24, -1.76 ppm and tetraphosphate **179f** $[M+H]^+$ 1491 (100), $[M+Na]^+$ 1513 (56); δ_P (162 MHz, $CDCl_3$) -1.53, -3.05 (2P), -7.09 ppm.

4-*C*-Methyl-*myo*-inositol 1,3,4,5-*O*-tetraphosphate (191a)



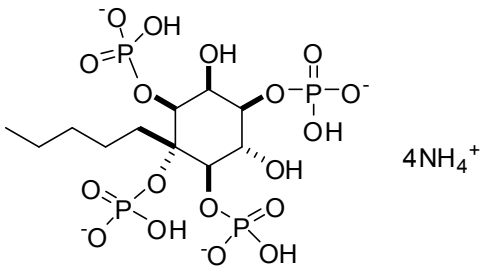
1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-methyl-*myo*-inositol (**179a**, 350 mg, 0.25 mmol) was hydrogenated according to general method 10 to yield the *title compound* (**191a**, 69 mg, 100 %); δ_H (500 MHz, D_2O) 4.23 (1H, t, *J* 3.4, Ins 2-***H***), 4.14 (1H, dd, *J* 10.1, 3.4, Ins 3-***H***), 4.02 (1H, t, *J* 9.5, Ins 5-***H***), 3.87 (1H, ddd *J* 10.1, 8.5, 3.3, Ins 1-***H***), 3.66 (1H, t, *J* 9.9, Ins 6-***H***), 1.44 (3H, s, Ins 4-C***H₃***) ppm; δ_C (125 MHz, D_2O) 84.05 (dt, *J* 6.8, 4.9, Ins 4-***C***CH_3), 81.28 (bm), 76.69 (d, *J* 5.7), 73.92 (d, *J* 5.2), 70.48 (s), 70.21 (bm) (5 × Ins ***C***H), 15.21 (s, Ins 4-***C***H_3) ppm; δ_P (162 MHz, D_2O) 1.74, 1.38, 0.83, -2.35 ppm; HRMS (ESI^-) *m/z* (%) found [M-$H]^-$ 512.9350 (77), $C_7H_{17}O_{18}P_4$ requires 512.9365.

4-*C*-Ethyl-*myo*-inositol 1,3,4,5-*O*-tetraphosphate (191b)



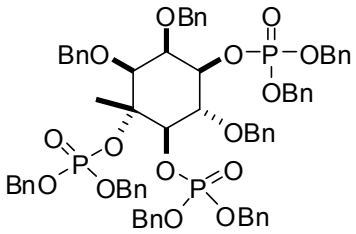
1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-ethyl-*myo*-inositol (**179b**, 160 mg, 0.11 mmol) was hydrogenated according to general method 6 to yield the *title compound* (**191b**, 59 mg, 100 %); δ_H (500 MHz, D_2O) 4.51 (1H, dd, *J* 9.3, 2.9, Ins 3-***H***), 4.37 (1H, t, *J* 9.3, Ins 5-***H***), 4.36 (1H, t, *J* 3.4, Ins 2-***H***), 3.89 (1H, td, *J* 9.3, 3.4, Ins 1-***H***), 3.77 (1H, t, *J* 8.8, Ins 6-***H***), 1.94-1.83 (2H, m, C***H₂***CH_3), 0.93 (3H, t, *J* 7.3, CH_2C***H₃***) ppm; δ_C (125 MHz, D_2O) 84.68 (quintet, *J* 4.8, Ins ***C***), 79.16-78.86 (m), 75.48 (d, *J* 6.4), 74.64 (d, *J* 4.8), 70.80 (d, *J* 6.4), 69.86-69.61 (m) (5 × Ins ***C***H), 24.95 (d, *J* 6.4, ***C***H_2CH_3), 8.94 (CH_2***C***H_3) ppm; δ_P (162 MHz, D_2O) 1.87, 0.71, 0.27, -4.03 ppm; HRMS (ES^-) *m/z* (%) found $[M-H]^-$ 526.9519 (100), $C_8H_{19}O_{18}P_4$ requires 526.9522.

4-*C*-Pentyl-*myo*-inositol 1,3,4,5-*O*-tetraphosphate (191c)



1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-pentyl-*myo*-inositol (**179c**, 95 mg, 0.06 mmol) was hydrogenated according to general method 10 to yield the *title compound* (**191c**, 40 mg, 94 %); δ_H (500 MHz, D_2O, 298 K) 4.59 (1H, dd *J* 9.5, 3.0, Ins 3-***H***), 4.45 (1H, t, *J* 9.3, Ins 5-***H***), 4.44 (1H, t, *J* 3.0, Ins 2-***H***), 3.97 (1H, dt, *J* 9.2, 3.6, Ins 1-***H***), 3.83 (1H, t, *J* 9.0, Ins 6-***H***), 1.96-1.91 (1H, m), 1.87-1.81 (1H, m), 1.61 (2H, quintet, *J* 7.8), 1.23 (2H, sextet, *J* 7.3), 1.18-1.13 (2H, m) (4 × C***H***$_2$), 0.79 (3H, t, *J* 7.4, C***H***$_3$) ppm; δ_C (125 MHz, D_2O, 298 K) 84.97 (q, *J* 4.9, Ins 4-***C***), 79.19-79.13 (m, Ins 5-***C***H), 75.61 (d, *J* 6.0, Ins 3-***C***H), 74.71 (d, *J* 5.4, Ins 1-***C***H), 70.97 (d, *J* 5.2, Ins 6-***C***H), 70.08-70.02 (m, Ins 2-***C***H) 32.56, 32.36 (d, *J* 7.1), 23.43, 21.94 (4 × ***C***H$_2$), 13.58 (***C***H$_3$) ppm; δ_P (162 MHz, D_2O, 298 K) 1.88, 0.65, 0.18, -4.10 ppm; HRMS (ES^-) *m/z* (%) found $[M-H]^-$ 568.9976 (100), $C_{11}H_{25}O_{18}P_4$ requires 568.9991.

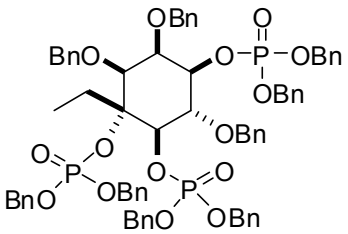
1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-methyl-*myo*-inositol (194a)



2,3,6-*O*-Tribenzyl-4-*C*-methyl-*myo*-inositol (**175a**, 64 mg, 0.14 mmol) was phosphitylated using *N,N*-diisopropyldibenzyl phosphoramidite and then oxidised with *m*CPBA as described in general method 6. The crude material was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (3:1 → 0:1 v/v) afforded the *title compound* (**194a**, 97 mg, 57 %); R_f (hexane-EtOAc, 7:3 v/v) 0.15; δ_H (400 MHz, $CDCl_3$) 7.42-7.02 (45H, m, 45 × Ar ***H***), 5.12-4.68 [18H, m, (17 × Ph-CH***H***O) + Ins 5-***H***], 4.58-4.50 (3 H, m, OCH***H***Ph + Ins 1-***H*** + Ins 2-***H***), 4.16 (1H, d, *J* 2.3, Ins 3-***H***), 4.00 (1H, t, *J* 9.6, Ins 6-***H***), 1.02 (3H, s, Ins 4-C***H***$_3$) ppm; δ_C (100 MHz, $CDCl_3$) 138.38, 138.21, 137.97 (3 × Ar ***C***), 136.26 (d, *J* 7.8), 136.10 (d, *J* 7.8), 136.03 (d, *J* 6.0), 135.81 (d, *J* 7.1), 135.60 (d, *J* 6.5), 135.57 (d, *J* 6.6) (6 × Ar ***C***CH$_2$OP), 128.56 (5C), 128.35 (5C), 128.29 (4C), 128.23 (5C), 128.13 (4C), 127.99 (3C), 127.92 (2C), 127.83 (2C), 127.76 (2C), 127.61 (2C), 127.58 (2C), 127.49, 127.44 (2C), 127.34, 127.27 (2C), 127.16 (3C) (45 × Ar ***C***H), 89.35 (dd, Ins 4-***C***CH3, *J* 8.2, 2.6), 82.73 (bs, Ins 5-***C***H), 78.40 (Ins 3-***C***H), 77.54 (d, *J* 5.6, Ins 1-***C***H), 77.09 (d, *J*

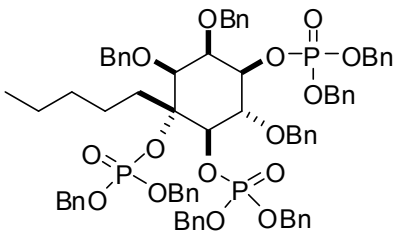
6.1, Ins 6-***C***H), 75.29 (2-O***C***H_2Ph), 74.59 (6-O***C***H_2Ph), 74.15 (Ins 2-***C***H), 70.84 (3-O***C***H_2Ph), 69.63 (d, *J* 5.2), 69.39 (d, *J* 5.2), 69.22 (2C, d, *J* 5.2), 69.03 (2C, d, *J* 5.1) (6 × PO***C***H_2Ph), 17.65 (d, *J* 2.1, Ins 4-***C***H_3) ppm; δ_P (162 MHz, $CDCl_3$) -1.88, -2.06, -7.23 ppm; HRMS (ES_+) *m/z* (%) found $[M+H]_+$ 1245.4048 (100), $C_{70}H_{72}O_{15}P_3$ requires 1245.4084.

1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-ethyl-*myo*-inositol (194b)



2,3,6-*O*-Tribenzyl-4-*C*-ethyl-*myo*-inositol (**175b**, 67 mg, 0.14 mmol) was phosphitylated using *N,N*-diisopropyldibenzyl phosphoramidite and then oxidised with *m*CPBA as described in general method 6. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:0 v/v) afforded the *title compound* (**194b**, 143 mg, 81 %); R_f (EtOAc-hexane, 3:7 v/v) 0.11; δ_H (400 MHz, $CDCl_3$) 7.41-7.00 (45H, m, 45 × Ar ***H***), 5.09-4.67 [18H, m, (17 × OCH***H***Ph) + Ins 5-***H***], 4.61-4.52 (3H, m, OCH***H***Ph + Ins 1-***H*** + Ins 2-***H***), 4.43 (1H, d, *J* 2.3, Ins 3-***H***), 4.08-4.02 (1H, m, Ins 6-***H***), 2.48 (1H, dqd, *J* 14.9, 7.4, 3.0, CH***H***CH_3), 2.18 (1H, dq, *J* 14.1, 7.4, CH***H***CH_3), 1.19 (3H, t, *J* 7.4, CH_2C***H***$_3$) ppm; δ_C (100 MHz, $CDCl_3$) 138.33, 138.26, 138.92 (3 × Ar ***C***), 136.27 (d, *J* 8.0), 136.11 (d, *J* 7.8), 136.03 (d, *J* 8.1), 135.88 (d, *J* 7.4), 135.70 (d, *J* 5.8), 135.64 (d, *J* 6.4) (6 × Ar ***C***CH_2OP), 128.53 (2C), 128.51 (2C), 128.42, 128.34 (3C), 128.29 (3C), 128.25 (2C), 128.17 (2C), 128.13 (4C), 128.08 (2C), 127.98, 127.91 (3C), 127.83 (2C), 127.78 (3C), 127.59 (2C), 127.50 (4C), 127.45 (2C), 127.32 (2C), 127.23, 127.16, 126.91 (3C) (45 × Ar ***C***H), 89.87-89.68 (m, Ins 4-***C***), 78.68, 77.63, 77.25 (2C) (4 × Ins ***C***H), 74.39 (2 × O***C***H_2Ph), 73.83 (Ins ***C***H), 69.50 (d, *J* 5.2), 69.34-69.10 (5C, m), 68.83 (d, *J* 5.9) [O***C***H_2Ph + (6 × PO***C***H_2Ph)], 25.82 (d, *J* 4.3, ***C***H_2CH_3), 9.36 (CH_2***C***H_3) ppm; δ_P (162 MHz, $CDCl_3$) -1.82, -2.15, -7.07 ppm; MS (ES^+) *m/z* (%) found $[M+H]^+$ 1259 (55), $[M+Na]^+$ 1281 (100).

1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-pentyl-*myo*-inositol (194c)



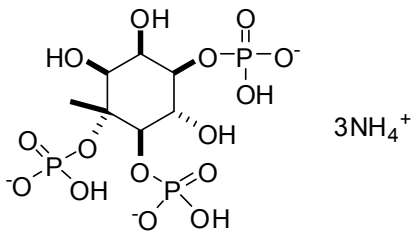
2,3,6-*O*-Tribenzyl-4-*C*-pentyl-*myo*-inositol (**175c**, 60 mg, 0.12 mmol) was phosphitylated using *N,N*-diisopropyldibenzyl phosphoramidite and then oxidised with *m*CPBA as described in

general method 6. The crude material was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (3:1 → 0:1 v/v) afforded the *title compound* (**194c**, 74 mg, 49 %); R_f (EtOAc-hexane, 3:7 v/v) 0.12; δ_H (400 MHz, $CDCl_3$) 7.44-6.99 (45H, m, 45 × Ar ***H***), 5.11 (2H, d, *J* 6.7), 5.01-4.55 (19H, m) (18 × OCH***H***Ph, Ins 1-***H***, Ins 2-***H***, Ins 5-***H***), 4.47 (1H, d, *J* 2.2, Ins 3-***H***), 4.10-4.02 (1H, m, Ins 6-***H***), 2.37 (1H, bt, *J* 12.4), 2.10 (1H, bt, *J* 13.1) [C***H₂***$(CH_2)_3CH_3$], 1.85-1.80 (1H, m), 1.75-1.66 (1H, m) [CH_2C***H₂***$(CH_2)_2CH_3$], 1.21-1.07 [4H, m, $(CH_2)_2$(C***H₂***$)_2CH_3$], 0.80 [3H, t, *J* 7.0, $(CH_2)_4$C***H₃***] ppm; δ_C (100 MHz, $CDCl_3$) 138.28 (2C), 138.23 (3 × Ar ***C***), 136.34 (d, *J* 6.9), 136.14 (d, *J* 8.7), 136.05 (d, *J* 8.7), 135.89 (d, *J* 8.7), 135.69 (d, *J* 6.9), 135.63 (d, *J* 5.2) (6 × ***C***CH_2OP), 128.52 (3C), 128.43, 128.31 (3C), 128.28 (3C), 128.23 (2C), 128.17 (3C), 128.12 (5C), 128.09 (4C), 128.03, 127.93 (3C), 127.79 (5C), 127.57 (2C), 127.50 (2C), 127.42 (2C), 127.28 (2C), 127.21, 127.12, 126.88 (2C) (45 × Ar ***C***H), 89.89-89.83 (m, Ins 4-***C***), 78.87, 77.64, 77.25 (3 × Ins ***C***H), 74.43 (2 × O***C***H_2Ph), 73.82 (2 × Ins ***C***H), 69.51 (d, *J* 5.2), 69.30 (d, *J* 5.2), 69.19-69.04 (3C, m), 68.74 (d, *J* 5.2) (6 × PO***C***H_2Ph), 33.18 (d, *J* 5.2), 32.76, 24.28-24.08 (m), 22.62 (4 × ***C***H_2), 14.05 (***C***H_3) ppm; δ_P (162 MHz, $CDCl_3$) -1.83, -2.19, -7.12 ppm; MS (ES^+) *m/z* (%) found $[M+H]^+$ 1301 (58), $[M+Na]^+$ 1323 (100).

* Third benzyl peak not visible

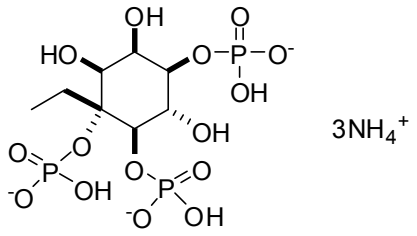
** Ins-CH assigned using ^1H-^{13}C correlation spectroscopy

4-*C*-Methyl-*myo*-inositol 1,4,5-*O*-triphosphate (195a)

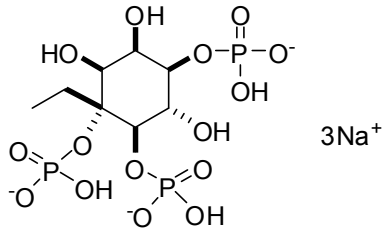


1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-methyl-*myo*-inositol (**194a**, 64 mg, 0.05 mmol) was hydrogenated according to general method 9. The powdery solid was re-dissolved in the minimum volume of H_2O and passed through DOWEX 50WX8-200 H^+ resin. Acidic fractions of eluent were combined, neutralised with aq. ammonium and freeze dried to yield the *title compound* (**195a**, 30 mg, 100 %) as a pale brown powdery salt; δ_H (400 MHz, D_2O) 4.20 (1H, t, *J* 3.1, Ins 2-***H***), 4.05 (1H, dd, *J* 9.5, 8.9, Ins 5-***H***), 3.98 (1H, ddd, *J* 9.6, 9.1, 3.0, Ins 1-***H***), 3.89 (1H, d, *J* 3.2, Ins 3-***H***), 3.75 (1H, dd, *J* 9.9, 9.8, Ins 6-***H***), 1.48 (3H, s, Ins 4-C***H₃***) ppm; δ_C (125 MHz, D_2O) 87.76 (d, Ins 4-***C***CH_3, *J* 7.0), 83.82 (t, *J* 6.8), 77.65 (d, *J* 5.3), 75.59 (s), 72.59 (s), 72.33 (s) (5 × Ins ***C***H), 17.81 (s, Ins 4-***C***H_3) ppm; δ_P (162 MHz, D_2O) 2.55, 2.31, 0.30 ppm; HRMS (ES^-) *m/z* (%) found $[M-H]^-$ 432.9716 (100), $C_7H_{16}O_{15}P_3$ requires 432.9702.

4-*C*-Ethyl-*myo*-inositol 1,4,5-*O*-triphosphate (195b)

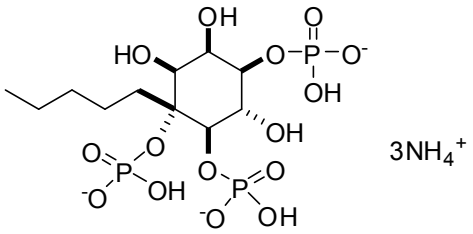


As the ammonium salt: 1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-ethyl-*myo*-inositol (**194b**, 138 mg, 0.11 mmol) was hydrogenated according to general method 9. The powdery solid was re-dissolved in the minimum volume of H_2O and passed through DOWEX 50WX8-200 H^+ resin. Acidic fractions of eluent were combined, neutralised with aq. ammonium and freeze dried to yield the *title compound* (49 mg, 100 %) as a pale brown powdery salt; δ_H (500 MHz, D_2O) ~4.48-4.70 (under HOD, Ins 2-***H*** + Ins 5-***H***), 4.55 (1H, d, *J* 2.7, Ins 3-***H***), 4.50 (1H, dt, *J* 8.4, 2.7, Ins 1-***H***), 4.41 (1H, t, *J* 7.8, Ins 6-***H***), 2.44 (1H, dt, *J* 22.0, 7.3), 2.34 (1H, dt, *J* 21.8, 7.3) (2 × CH***H***CH_3), 1.44 (3H, t, *J* 7.3, CH_2C***H₃***) ppm; δ_P (162 MHz, D_2O) 1.34, 0.58, -3.25 ppm; HRMS (ES^-) *m/z* (%) found $[M-H]^-$ 446.9842 (100), $C_8H_{18}O_{15}P_3$ requires 446.9859.



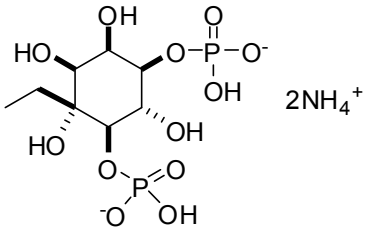
As the sodium salt: 4-*C*-Ethyl-*myo*-inositol 1,4,5-*O*-triphosphate ammonium salt (45 mg, 0.09 mmol) was stirred with DOWEX 50WX8-200 H^+ resin which had been treated with NaOH. After filtration, the mother liquor was freeze dried to yield the *title compound* (47 mg, 100 %); δ_H (500 MHz, D_2O) 4.61 (1H, t, *J* 3.4, Ins 2-***H***), 4.48 (1H, t, *J* 8.2, Ins 5-***H***), 4.40-4.37 (1H, m, Ins 3-***H***), 4.34-4.29 (1H, m, Ins 1-***H***), 4.27-4.23 (1H, m, Ins 6-***H***), 2.34 (1H, dt, *J* 21.9, 7.3), 2.22 (1H, dt, *J* 21.9, 7.2) (2 × CH***H***CH_3), 1.31 (3H, t, *J* 7.3, CH_2C***H₃***) ppm; δ_C (125 MHz, D_2O) 83.86-83.76 (m, Ins 4-***C***), 78.61-78.10 (br m, Ins 5-***C***H), 75.45 (d, *J* 4.4, Ins 1-***C***H), 73.13 (d, *J* 5.5, Ins 3-***C***H), 72.22 (d, *J* 5.5, Ins 6-***C***H), 69.31-69.06 (m, Ins 2-***C***H), 24.96 (***C***H_2CH_3), 9.07 (CH_2***C***H_3) ppm; δ_P (162 MHz, D_2O) 4.45, 4.08, 0.52 ppm: HRMS (ES^-) *m/z* (%) found $[M-H]^-$ 446.9842 (100), $C_8H_{18}O_{15}P_3$ requires 446.9859.

4-*C*-Pentyl-*myo*-inositol 1,4,5-*O*-triphosphate (195c)



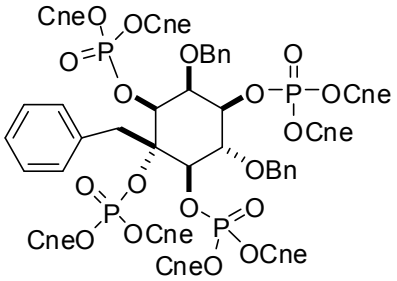
1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-pentyl-*myo*-inositol (**194c**, 80 mg, 0.06 mmol) was hydrogenated according to general method 9. The powdery solid was re-dissolved in the minimum volume of H_2O and passed through DOWEX 50WX8-200 H^+ resin. Acidic fractions of eluent were combined, neutralised with aq. ammonium and freeze dried to yield the *title compound* (**195c**, 30 mg, 100 %) as a pale brown powdery salt; δ_H (500 MHz, D_2O, 318 K) 4.56-4.52 (2H, m), 4.39-4.36 (1H, m), 4.24-4.17 (1H, m), 4.15-4.08 (1H, m) (5 × Ins ***H***), 2.15-1.98 (2H, m), 1.86-1.77 (2H, m), 1.50-1.39 (4H, m) (4 × C***H***$_2$), 1.04 (3H, t, *J* 7.3, C***H***$_3$) ppm; δ_C (125 MHz, D_2O, 318 K) 84.82-84.66 (m, Ins ***C***), 78.24-78.06 (m), 75.24 (d, *J* 5.0), 72.42, 71.66, 70.33-69.93 (m) (5 × Ins ***C***H), 32.70, 32.66, 23.38, 22.25 (4 × ***C***H_2), 13.78 (***C***H_3) ppm; δ_P (162 MHz, D_2O, 318 K) 3.11, 2.61, -2.38 ppm; HRMS (ES^-) *m/z* (%) found $[M-H]^-$ 489.0315 (100), $C_{11}H_{24}O_{15}P_3$ requires 489.0328.

4-*C*-Ethyl-*myo*-inositol 1,5-*O*-diphosphate (196b)



From decomposition of 4-*C*-ethyl-*myo*-inositol 1,4,5-*O*-triphosphate (195b): δ_H (500 MHz, D_2O) 4.61 (1H, t, *J* 3.4, Ins 2-***H***), 4.53 (1H, dt, *J* 8.1, 3.4, Ins 1-***H***), 4.46-4.41 (1H, m, Ins 6-***H***), 4.31 (1H, t, *J* 8.7, Ins 5-***H***), 4.03 (1H, d, *J* 3.4, Ins 3-***H***), 2.28 (1H, dt, *J* 22.4, 7.6), 2.17 (1H, dt, *J* 22.4, 7.6) (2 × CH***H***CH_3), 1.37 (3H, t, *J* 7.6, CH_2C***H***$_3$) ppm; δ_C (125 MHz, D_2O) 80.77-80.54 (m, Ins 5-***C***H), 77.20 (d, *J* 3.3, Ins 4-***C***), 76.20 (d, *J* 5.5, Ins 1-***C***H), 74.68 (Ins 3-***C***H), 70.72 (t, *J* 3.3, Ins 6-***C***H), 69.66-69.43 (m, Ins 2-***C***H), 25.37 (***C***H_2CH_3), 8.02 (CH_2***C***H_3) ppm; δ_P (162 MHz, D_2O) 1.09, 0.77 ppm; HRMS (ESI^-) *m/z* (%) found $[M-H]^-$ 367.0184 (100), $C_8H_{17}O_{12}P_2$ requires 367.0195.

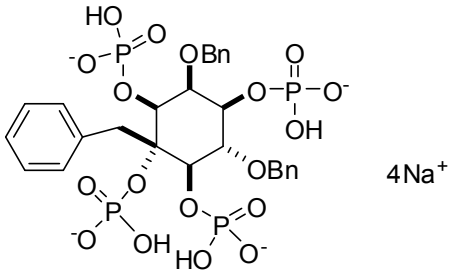
1,3,4,5-*O*-Tetrakis(dicyanoethylphosphoryl)-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol (197)



2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol (**151f**, 200 mg, 0.44 mmol) and 3-nitro-1,2,4-1H-triazole (709 mg, 6.22 mmol) were evaporated from MeCN (3 × 2 mL) and taken up in pyridine (2 mL). Dicyanoethyl phosphorochloridite (0.5 mmol/mL solution in CH_2Cl_2, 6.22 mL, 3.11 mmol) was added and the solution stirred for 2 h. After addition of cyanoethyl (88 µL, 1.33 mmol) the reaction was stirred for a further 1 h, before cooling to 0 ºC. *m*CPBA (75 %, 1.07 g, 6.22 mmol) was added portion-wise and the resulting solution allowed to warm to rt and stirred for a further 2 h. The reaction was quenched with $Na_2S_2O_3$ (10 % solution in H_2O) and stirred for 30 min before extracting with CH_2Cl_2 (4 × 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude residue (1.60 g) was separated from phosphorylating reagent by chromatography on reverse phase silica. Elution with MeCN-H_2O (1:19 → 1:1 v/v) afforded the crude product which was then fractionated by chromatography on flash silica. Elution with MeOH-CH_2Cl_2 (0:1 → 3:50 v/v) afforded the *title compound* (**197**, 127 mg, 24 %) as a clear oil; R_f (MeOH-CH_2Cl_2, 1:25 v/v) 0.37; δ_H (400 MHz, $CDCl_3$) 7.51-7.46 (4H, m), 7.42-7.36 (5H, m), 7.35-7.25 (5H, m), 7.23-7.19 (1H, m) (15 × Ar ***H***), 5.37-5.34 (1H, m, Ins ***H***), 5.06-5.04 (1H, m, Ins ***H***), 5.04 (1H, d, *J* 12.7), 5.00 (1H, d, *J* 11.4), 4.97 (1H, d, *J* 11.0), 4.89 (1H, d, *J* 11.0) (4 × OCH***H***Ph), 4.78-4.72 (2H, m, 2 × Ins ***H***), 4.27-3.95 [17H, m, (8 × OC***H₂***CH_2CN) + Ins ***H***], 3.65 (1H, dd, *J* 15.0, 3.5), 3.47 (1H, d, *J* 15.4) (2 × CCH***H***Ph), 2.76-2.54 (16H, m, 8 × OCH_2C***H₂***CN) ppm; δ_C (100 MHz, $CDCl_3$) 137.79, 137.57 (2 × Ar ***C***)*, 131.82, 128.66 (3C), 128.56 (3C), 128.16 (2C), 128.04, 127.90 (2C), 127.84, 127.54, 126.63 (15 × Ar ***C***H), 117.12, 117.00, 116.80, 116.69, 116.64, 116.51 (2C), 116.43 (8 × OCH_2CH_2***C***N), 88.06-87.94 (m, Ins ***C***), 77.21, 77.15, 77.10, 77.00, 76.91 (5 × Ins ***C***H), 76.21, 74.89 (2 × O***C***H_2Ph), 63.06-62.96 (4C, m), 62.87 (d, *J* 5.5), 62.83 (d, *J* 4.9), 62.74 (d, *J* 4.9), 62.70 (d, *J* 4.9) (8 × O***C***H_2CH_2CN), 19.63 (d, *J* 7.6), 19.60 (d, *J* 8.2), 19.57 (d, *J* 6.5), 19.54 (2C, d, *J* 8.2), 19.52 (2C, d, *J* 7.6), 19.31 (d, *J* 7.6) (8 × OCH_2***C***H_2CN) ppm; δ_P (162 MHz, $CDCl_3$) -3.17, -3.99, -4.17, -8.61 ppm; MS (ES^+) *m/z* (%) found 338 (100), $[M+H]^+$ 1195 (61), $[M+Na]^+$ 1217 (92).

* Third Ar ***C*** peak not visible in ^{13}C-NMR spectra.

2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,4,5-*O*-tetraphosphate (198)

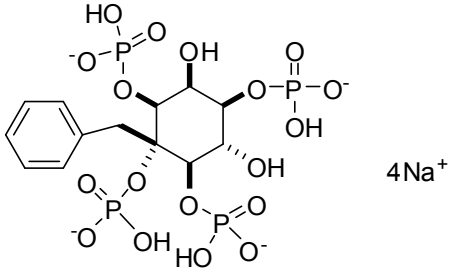


1,3,4,5-*O*-Tetrakis(dicyanoethylphosphoryl)-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol (**197**, 77 mg, 0.06 mmol) was evaporated from MeCN (3 × 1 mL) and then taken up in MeCN (1 mL). Barton's base (155 μL, 0.77 mmol) and TmsCl (82 μL, 0.65 mmol) were added and the solution stirred for 16 h. All solvents were evaporated under reduced pressure and the residue was evaporated from toluene (2 × 2 mL). The residue was washed with ether-hexane (1:1 v/v) and filtered under N_2 using the Gaffney filtration apparatus. The filtrate was evaporated to dryness and then stirred with $NaHCO_3$ (20 mg, 0.24 mmol) in MeOH (2 mL) . After 10 mins, all solvents were evaporated and the residue evaporated from MeCN (2 × 5 mL), to afford the *title compound* (**198**, 47 mg, 85 %) as a white residue; δ_H (500 MHz, MeOD) 7.68 (2H, d, *J* 7.3), 7.53 (2H, d, *J* 7.3), 7.34 (6H, t, *J* 7.3), 7.26 (2H, t, *J* 6.8), 7.09 (2H, t, *J* 6.8), 7.03 (1H, t, *J* 6.8) (15 × Ar ***H***), 5.04 (1H, t, *J* 8.3, Ins 3-***H***), 5.01 (2H, s, OC***H*****2**Ph), 4.98 (1H, d, *J* 10.5), 4.95 (1H, d, *J* 10.5) (2 × OCH***H***Ph), ~4.94 (under HOD, Ins 5-***H***), 4.72 (1H, t, *J* 3.4, Ins 2-***H***), 4.37 (1H, t, *J* 8.3, Ins 1-***H***), 4.06 (1H, t, *J* 9.8, Ins 6-***H***), 3.55 (1H, d, *J* 14.2), 3.31 (1H, d, *J* 14.2) (2 × CCH***H***Ph) ppm; δ_C (100 MHz, MeOD) 139.50, 138.39, 138.06 (3 × Ar ***C***), 131.77 (2C), 129.45 (2C), 127.66 (4C), 127.57 (2C), 127.13, 126.77, 126.40 (2C), 124.58 (15 × Ar ***C***H), 79.32 (Ins 5-***C***H), 78.71 (Ins 2-***C***H), 77.27 (Ins 6-***C***H), 76.08 (O***C***H_2Ph), 75.57 (Ins 1-***C***H), 75.33 (Ins 3-***C***H), 74.87 (O***C***H_2Ph), 36.79 (C***C***H_2Ph) ppm; δ_P (162 MHz, D_2O) 0.13, -0.17, -0.23, -2.99 ppm; HRMS (ESI$^-$) *m/z* (%) found $[M-H]^-$ 769.0649 (65), $C_{27}H_{33}O_{18}P_4$ requires 769.0617.

*Contaminated with Bartons Base.

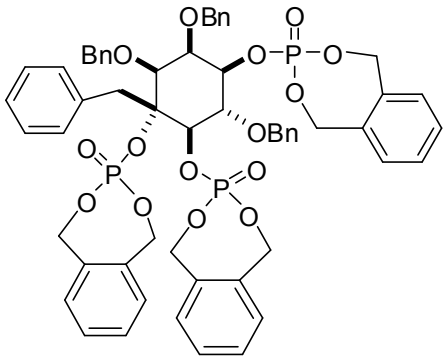
Quatenary 4-*C* not visible in ^{13}C-NMR spectrum.

4-*C*-Benzyl-*myo*-inositol 1,3,4,5-*O*-tetraphosphate.



2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,4,5-*O*-tetraphosphate (**198**, 23 mg, 0.03 mmol) was taken up in ^{t}BuOH-H_2O (6:1 v/v, 3 mL) to which was added Pd-black (18 mg, 0.16 mmol). The solution was stirred under an atmosphere of H_2 for 36 h. The catalyst was filtered off, washed with H_2O (4 × 10 mL) and the filtrate concentrated under reduced pressure. The remaining solution was taken up in H_2O, washed with CH_2Cl_2 (× 2) and freeze dried. The powdery solid was re-dissolved in the minimum volume of H_2O and passed through DOWEX 50WX8-200 H^+ resin. Acidic fractions of eluent were combined, neutralised with aq. NH_3 and freeze dried to afford the *title compound* (13 mg, 72 %) as a brown powdery salt; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 590.9850, $C_{13}H_{23}O_{18}P_4$ requires 590.9835 and $[M+Bn+H]^+$ 681.0322, $C_{20}H_{29}O_{18}P_4$ requires 681.0304.

1,4,5-*O*-Tris(5,6-benzo-1,3,2-dioxaphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol (202)

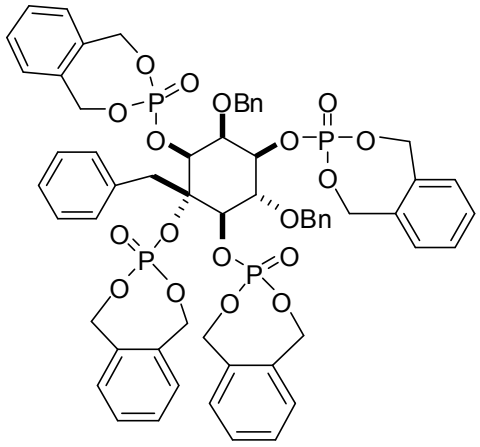


2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol (**175f**, 79 mg, 0.15 mmol) and 1-*H* tetrazole (102 mg, 1.46 mmol) were evaporated from MeCN (3 × 1 mL) and taken up in MeCN (1 mL). The resulting suspension was placed in a water bath at 25 ºC, and *N,N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane (158 μL, 1.02 mmol) added, upon which all solids dissolved immediately. After 2 h the solution was cooled to 0 ºC and *m*CPBA (336 mg, 1.46 mmol) added. The solution was then allowed to warm to rt and stirred for a further 1 h 30 min, before addition of $Na_2S_3O_3$ (10 % solution in H_2O). After 30 min the organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (× 3). The combined organic layers were washed with brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 1:0 v/v) afforded the *title compound* (**202**, 69 mg, 44 %) as a clear oil; R_f (EtOAc-hexane, 3:7 v/v) 0.19; δ_H (400 MHz, $CDCl_3$) 7.52-7.47 (4H, m), 7.35-6.95 (28H, m) (32 × Ar ***H***), 5.30-4.74 (17H, m, 3 × Ins ***H***, 3 × OC***H***$_2$Ph, 8 × OCH***H***Ar), 4.65-4.60 (3H, m, Ins ***H***, OCH_2Ar), 4.39 (1H, dd, *J* 13.7, 9.8, OCH***H***Ar), 4.27-4.21 (Ins ***H***), 4.09-4.06 (1H, m, OCH***H***Ar), 4.00 (1H, d, *J* 13.7), 3.61 (1H, d, *J* 11.7) (2 × CCH***H***Ph) ppm; δ_C (100 MHz, $CDCl_3$) 138.22, 137.80, 137.68, 135.71, 135.58 (2C), 135.45, 135.19, 135.09, 132.57 (10 × Ar ***C***), 129.17, 129.08 (2C), 129.02 (2C), 128.98 (2C), 128.77 (3C),

128.66 (2C), 128.60 (2C), 128.49 (2C), 128.40 (3C), 128.06 (4C), 127.74 (2C), 127.69, 127.53 (2C), 127.43 (2C), 127.29, 127.26 (32 × Ar ***C***), 88.19-88.15 (m, Ins ***C***), 82.84-82.74 (m), 78.93, 77.25, 76.52-76.46 (m) (4 × Ins ***C***H), 76.26, 75.68 (2 × O***C***H_2Ph), 74.72-74.67 (m, Ins ***C***H), 71.91 (O***C***H_2Ph), 68.89 (d, *J* 6.4), 68.57 (d, *J* 11.2), 68.52 (d, *J* 11.2), 68.34 (d, *J* 6.4), 68.18 (d, *J* 6.4), 68.10 (d, *J* 9.6) (6 × O***C***H_2Ar), 36.51 (C***C***H_2Ph) ppm; δ_P (162 MHz, D2O) -0.48, -2.27, -9.11 ppm; MS (ES+) *m/z* (%) found $[M+H]^+$ 1087 (40), $[M+Na]^+$ 1109 (100).

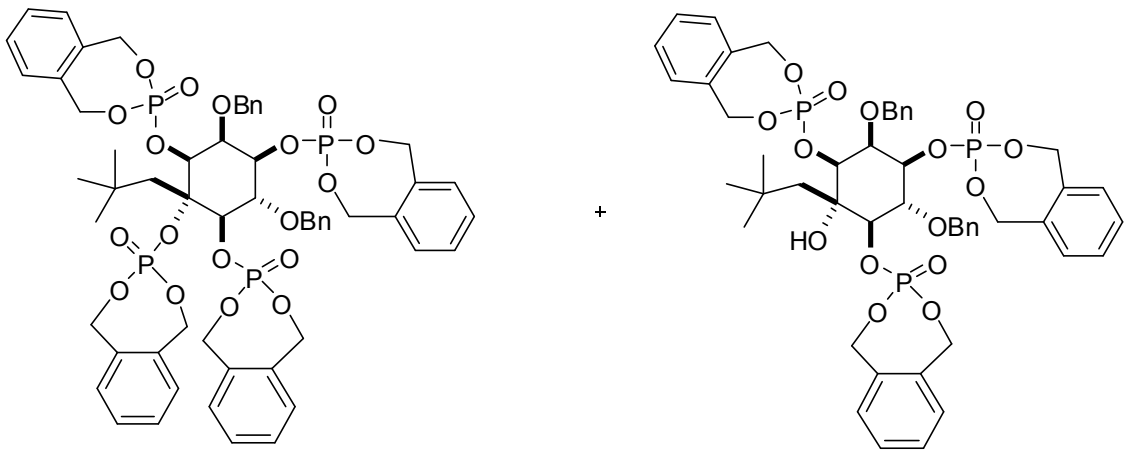
*****Inositol region assigned using ^{1}H-^{13}C correlation spectra.

1,3,4,5-*O*-Tetrakis(5,6-benzo-1,3,2-dioxaphosphoryl)-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol.



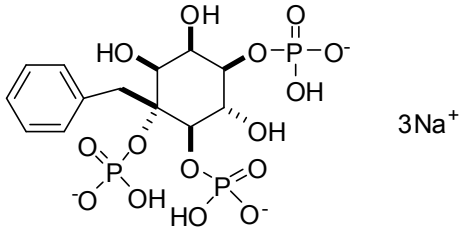
2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol (160 mg, 0.36 mmol) was phosphorylated according to general method 8. The crude material was not separated from reagent debris during purification using chromatography on flash silica. HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 1179.2699 (61), $C_{59}H_{59}O_{18}P_4$ requires 1179.2652, $[M+Na]^+$ 1201.2593 (100).

1,3,4,5-*O*-Tetrakis(5,6-benzo-1,3,2-dioxaphosphoryl)-2,6-*O*-dibenzyl-4-*C*-neopentyl-*myo*-inositol and 1,3,5-*O*-Tris(5,6-benzo-1,3,2-dioxaphosphoryl)-2,6-*O*-dibenzyl-4-*C*-neopentyl-*myo*-inositol.



2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol (65 mg, 0.15 mmol) was phosphorylated according to general method 8. On attempted fractionation of the crude material only a small fraction of triphosphate was isolated from phosphorylating reagent debris using chromatography on flash silica. For the triphosphate: δ_P (162 MHz, D2O) 0.99, -0.76, -1.98 ppm; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$977.2829 (83), $C_{49}H_{56}O_{15}P_3$ requires 977.2832, $[M+Na]^+$ 999.2631 (100)

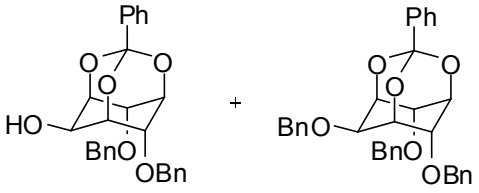
4-*C*-Benzyl-*myo*-inositol 1,4,5-*O*-triphosphate (203)



1,4,5-*O*-Tris(5,6-benzo-1,3,2-dioxaphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol (**202**, 64 mg, 0.06 mmol) was hydrogenated according to general method 9. The crude material (30 mg) was purified by HPLC. Elution with a gradient of H_2O-MeCN (49:1 → 1:1 v/v) yielded the *title compound* (**203**, 8 mg, 27 %) as a pale brown powdery salt; δ_H (500 MHz, D_2O) 7.44 (2H, d, *J* 7.5), 7.22 (2H, t, *J* 7.4), 7.14 (1H, t, *J* 7.3) (5 × Ar ***H***), 4.41-4.33 (1H, m), 4.30-4.27 (1H, m), 4.20-4.15 (1H, m), 4.08-3.99 (2H, m) (5 × Ins ***H***), 3.21 (2H, s, C***C***HPh) ppm; δ_P (162 MHz, D_2O) 0.91, 0.11, -4.33 ppm; HRMS (ES^-) *m/z* (%) found $[M-H]^-$ 508.9998 (100), $C_{13}H_{20}O_{15}P_3$ requires 509.0015.

7.6 Chapter 6

4,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (214) and 2,4,6-O-tribenzyl-*myo*-inositol 1,3,5-O-orthobenzoate (162)



Direct from orthobenzoate: *myo*-Inositol 1,3,5-*O*-orthobenzoate (**22d**, 550 mg, 2.04 mmol) was evaporated from MeCN (3 × 10 mL) and then toluene (3 × 5 mL). The residue was taken up in THF (15 mL) and cooled to 0 °C. *n*-BuLi (2.5 M in hexanes, 1.7 mL, 4.28 mmol) was added drop-wise and the solution allowed to warm to rt. BnBr (540 µL, 4.49 mmol) was taken up in DMF (4 mL) and added drop-wise to the reaction mixture. When complete by TLC, H_2O was added drop-wise and the volume reduced under reduced pressure. The product was taken up in EtOAc, washed with H_2O (×4) and then brine, dried ($MgSO_4$) and all solvents removed under

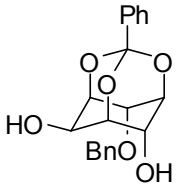
reduced pressure. The crude product was purified by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 2:5 v/v) afforded 4,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **214** (120 mg, 13 %) as a clear oil and 2,4,6-*O*-tribenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **162** (169 mg, 13 %) as a pale yellow oil.

From monobenzyl 215: 4-*O*-Benzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**215**, 1.38 g, 3.87 mmol) was evaporated from MeCN (3 × 10mL) and then toluene (3 × 5mL). The residue was taken up in THF (40 mL) and cooled to 0 °C. *n*-BuLi (2.5 M in hexanes, 1.54 mL, 3.87 mmol) was added drop-wise and the solution warmed slowly to 30 °C. BnBr (506µL, 3.87 mmol) was taken up in DMF (10 mL) and added drop-wise to the reaction mixture which was stirred at rt. When complete by TLC, the reaction was quenched by drop-wise addition of H_2O and the volume reduced under reduced pressure. The crude material was taken up in EtOAc, washed with H_2O (×4) and then brine, dried ($MgSO_4$) and all solvents removed under reduced pressure. The crude material was purified by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 1:1 v/v) afforded 4,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**214**, 716 mg, 41 %) and 2,4,6-*O*-tribenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**162**, 103 mg, 6 %) both as yellow oils.

4,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **214**: R_f (EtOAc-hexane, 1:1 v/v) 0.71; δ_H (400 MHz, $CDCl_3$) 7.66-7.36 (15H, m, Ar ***H***), 4.71 (2H, d, *J* 11.5, 2 × OCH***H***Ph), 4.64 (2H, d, *J* 11.5, 2 × OCH***H***Ph), 4.59 (1H, tt, *J* 3.4, 1.7, Ins 5-***H*)**, 4.51 (2H, t, *J* 4.0, Ins 4-***H*** + Ins 6-***H***), 4.45 (2H, dt, *J* 3.6, 1.8, Ins 1-***H*** + Ins 3-***H***), 4.29 (1H, m, Ins 2-***H***) ppm; δ_C (100 MHz, $CDCl_3$) 137.45 (2C), 136.82 (3 × Ar ***C***), 129.61, 128.42 (4C), 128.05 (2C), 127.86 (2C), 127.63 (4C), 125.15 (2C) (15 × Ar ***C***H), 107.91 (Ph***C***O_3), 74.24 (2C), 73.57 (2C) (4 × Ins ***C***H), 71.70 (2 × O***C***H_2Ph), 68.58, 60.62 (2 × Ins ***C***H) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 447 (56), $[M+NH_4]^+$ 461(9); HRMS (CI^+) found 447.1813, $C_{27}H_{27}O_6$ requires 447.1808.

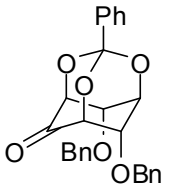
2,4,6-*O*-Tribenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **162**: R_f (EtOAc-hexane, 1:1 v/v) 0.76; δ_H (270 MHz, $CDCl_3$) 7.67-7.63 (2H, m), 7.35-7.24 (18H, m) (20 × Ar ***H***), 4.68 (2H, s, OC***H₂***Ph), 4.64 (2H, d, *J* 11.6, 2 × OCH***H***Ph), 4.56-4.44 (5H, m, 5 × Ins ***H***), 4.51 (2H, d, *J* 11.6, 2 × OCH***H***Ph), 4.14-4.12 (1H, m, Ins ***H***) ppm; δ_C (100 MHz, $CDCl_3$) 138.10, 137.70 (2C), 137.21 (4 × Ar ***C***), 129.47, 128.48 (5C), 128.13 (3C), 128.01 (3C), 127.88 (2C), 127.69 (4C), 125.45 (2C) (20 × Ar ***C***H), 107.93 (Ph***C***O_3), 74.07 (2C), 71.98 (2C) (4 × Ins ***C***H), 71.68 (2C), 71.28 (3 × O***C***H_2Ph), 69.11, 66.20 (2 × Ins ***C***H) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 537 (100), $[M+NH_4]^+$ 551 (8).

4-*O*-Benzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (215)



myo-Inositol 1,3,5-*O*-orthobenzoate (**22d**, 1.0 g, 3.76 mmol) was evaporated from MeCN (3 × 5 mL) and taken up in DMF (20 mL). The solution was cooled to -15 °C. NaH (60 % in oil, 150 mg, 3.76 mmol) was added portion-wise and the solution stirred for 15 min before being allowed to warm to rt and stirred for a further 30 min. BnBr (434 mL, 3.76 mmol) was added drop-wise and the reaction mixture stirred for 4 h. The reaction was quenched at 0 °C by drop-wise addition of H_2O and the product taken up in EtOAc. The organic layer was washed with H_2O then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude product was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 1:1 v/v) afforded the *title compound* (**215**, 1.32 g, 99 %) as a colourless oil: R_f (EtOAc-hexane, 3:1 v/v) 0.72; δ_H (270 MHz, $CDCl_3$) 7.66-7.34 (10H, m, 10 × Ar ***H***), 4.73 (1H, d, *J* 14.2, OCH***H***Ph), 4.67 (1H, d, *J* 14.2, OCH***H***Ph), 4.60-4.52 (2H, m, 2 × Ins ***H***), 4.46-4.39 (3H, m, 3 × Ins ***H***), 4.18-4.11 (1H, m, Ins ***H***), 3.75 (1H, d, *J* 10.1, Ins O***H***), 3.16 (1H, d, *J* 11.8, Ins O***H***) ppm; HRMS (ESI^+) found 357.1324 (100), $C_{20}H_{21}O_6$ requires 357.1338.

4,6-*O*-Dibenzyl-inos-2-ose 1,3,5-*O*-orthobenzoate (216) and 4,6-*O*-dibenzyl-2,2-dihydroxy-inositol 1,3,5-*O*-orthobenzoate (217)

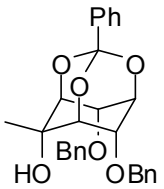


4,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**214**, 100 mg, 0.22 mmol) was evaporated from MeCN (3 × 1 mL) and taken up in CH_2Cl_2 (1 mL). DMP (180 mg, 0.44 mmol) was added portion-wise and the reaction stirred at rt for 3 h. The reaction was quenched by stirring with sat. $NaHCO_3$ and $Na_2O_3S_2.H_2O$ for 30 min. The product was taken up in CH_2Cl_2 and the organic layer washed with sat. $NaHCO_3$, H_2O and then brine, dried ($MgSO_4$), and all solvents removed under reduced pressure. The crude material was dried over molecular sieves in $CHCl_3$ for 24 h to afford 4,6-*O*-dibenzyl-inos-2-ose 1,3,5-*O*-orthobenzoate (**216**, 98 mg, 98 %) as a pale yellow oil and used without further purification: R_f (EtOAc-hexane, 3:7) 0.59; δ_H (400 MHz, $CDCl_3$) 7.67-7.65 (2H, m), 7.46-7.25 (13H, m) (15 × Ar ***H***), 4.77-4.74 (3H, m, 3 × Ins ***H***), 4.71 (2H, d, *J* 11.6), 4.67 (2H, d, *J* 11.6) (2 × OC***H₂***Ph), 4.61-4.59 (2H, m, 2 × Ins ***H***) ppm; δ_C (100 MHz, $CDCl_3$) 199.98 (Ins ***C***=O), 136.94 (2C), 135.24 (3 × Ar ***C***), 129.97, 128.54 (4C), 128.21

(2C), 128.08 (2C), 127.88 (4C), 125.5 (2C) (15 × Ar ***C***H), 108.18 (Ph***C***O_3), 78.44 (2C), 76.68 (2C) (4 × Ins ***C***H), 71.67 (2 × O***C***H_2Ph), 69.72 (Ins ***C***H) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 445 (42), $[M+NH_4]^+$ 462 (16); HRMS (ESI^+) found 445.1644 (58), $C_{27}H_{25}O_6$ requires 445.1651.

4,6-*O*-dibenzyl-2,2-dihydroxy-*myo*-inositol 1,3,5-*O*-orthobenzoate (**217**) was observed in the crude material and could be generated on addition of water to the inos-2ose **216**: R_f (EtOAc-hexane, 3:7) 0.85; δ_H (400 MHz, DMSO + D_2O) 7.59-7.24 (15H, m, 15 × Ar ***H***), 5.02 (1H, m, Ins ***H***), 4.75-4.73 (2H, m, 2 × Ins ***H***), 4.69-4.68 (2H, m, 2 × Ins ***H***), 4.64-4.63 (4H, m, 2 × OC***H***$_2$Ph) ppm.

2-*C*-Methyl-4,6-*O*-dibenzyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (218)



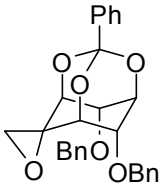
Directly from the inos-2ose: 4,6-*O*-Dibenzyl-inos-2-ose 1,3,5-*O*-orthobenzoate (**216**, 150 mg, 0.34 mmol) was evaporated from MeCN (3 × 1 mL) and taken up in ether (1 mL). The solution was cooled to -78 ºC and methylmagnesium bromide (135 µL, 0.41 mmol) added drop-wise. After 30 min at -78 ºC the solution was allowed to warm to rt and stirred 3 h. The reaction was quenched by drop-wise addition of H_2O and taken up in CH_2Cl_2. The organic layer was washed with 0.1 M HCl, sat. $NaHCO_3$, H_2O and then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was purified by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 3:7 v/v) afforded the *title compound* (**218**, 58 mg, 37 %) and unreacted starting material (**216**, 74 mg, 49 %).

From the *exo*-methylene oxide: 4,6-*O*-Dibenzyl-2,2-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**219**, 170 mg, 0.38 mmol) was evaporated from MeCN (3 × 1 mL) and taken up in THF (0.5 mL). $LiAlH_4$ (87 mg, 2.29 mmol) was taken up in THF (0.5 mL) and added to the methylidene solution, which was stirred for a further 3 h. The reaction was quenched by drop-wise addition of EtOAc and the crude material was taken up in ether. The organic layer was washed with sat. $NaHCO_3$, H_2O then brine, dried ($MgSO_4$) and all solvents removed under reduced pressure. The crude material (117 mg) was purified by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 1:5 v/v) afforded the *title compound* (**218**, 115 mg, 67 %) as a glass.

2-*C*-Methyl-4,6-*O*-dibenzyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**218**): R_f (EtOAc-hexane, 3:7 v/v) 0.74; δ_H (400 MHz, $CDCl_3$) 7.66-7.63 (2H, m), 7.40-4.37 (3H, m), 7.32-7.28 (10H, m) (15 × Ar ***H***), 4.77-4.74 (1H, m, Ins 5-***H***), 4.75 (2H, d, *J* 11.2), 4.70 (2H, d, *J* 11.2) (4 × OCH***H***Ph), 4.60 (2H, t, *J* 3.4, Ins 4-***H*** + Ins 6-***H***), 4.28 (2H, dt, *J* 3.9, 1.9, Ins 3-***H*** + Ins 1-***H***), 1.67

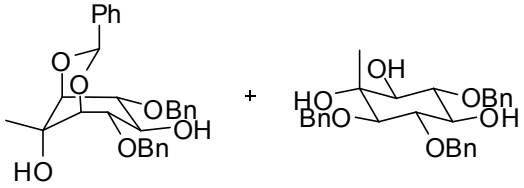
(Ins 4-CC***H₃***) ppm; δ_C (100 MHz, $CDCl_3$) 136.89 (2C), 136.59 (3 × Ar ***C***), 129.57, 128.54 (4C), 128.12 (2C), 128.04 (2C), 127.92 (4C), 125.32 (2C) (15 × Ar ***C***H), 107.5 (Ph***C***O_3), 74.19 (2C), 74.15 (2C) (4 × Ins ***C***H), 71.83 (2 × O***C***H_2Ph), 68.46 (Ins ***C***H), 67.83 (Ins ***C***), 24.99 (Ins 4-C***C***H_3) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 461 (100), $[M+NH_4]^+$ 478 (26); HRMS (ESI^+) found $[M+H]^+$ 461.1946 (63), $C_{28}H_{29}O_6$ requires 461.1964

4,6-*O*-Dibenzyl-2,2-*O*,*C*-methylidene-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (219)



To a clear solution of trimethylsulfoxonium iodide (130 mg, 0.59 mmol) in DMSO (2 mL) was added NaH (60% dispersion in mineral oil, 26 mg, 0.65 mmol) and the mixture stirred for 40 min. 4,6-*O*-Dibenzyl-inos-2-ose 1,3,5-*O*-orthobenzoate (**216**, 240 mg, 0.54 mmol) was evaporated from MeCN (2 mL × 3), taken up in THF (2 mL) and added to the reaction mixture, which was stirred at rt for 1 h. The reaction was quenched by drop-wise addition of H_2O (2 mL) and taken up in CH_2Cl_2. The organic layer was washed with sat. $NaHCO_3$, H_2O, then brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was fractionated using chromatography on flash silica. Elution with ether-hexane (0:1 → 3:5 v/v) afforded the *title compound* (**219**, 169 mg, 68 %) as a clear oil; R_f (ether- hexane, 7:3 v/v) 0.69; δ_H (400 MHz, $CDCl_3$) 7.67-7.65 (2H, m), 7.43-7.39 (7H, m), 7.36-7.28 (5H, m) (15 × Ar ***H***), 4.74-4.72 [5H, m, (2 × OCH***H***Ph) + Ins 5-***H***], 4.50 (2H, t, *J* 3.6, Ins 4-***H*** + Ins 6-***H***), 4.53 (2H, dt, *J* 3.4, 1.5, Ins 1-***H*** + Ins 3-***H***), 2.89 (2H, s, Ins 4-CC***H₂***) ppm; δ_C (100 MHz, $CDCl_3$) 129.79 (2C), 129.69 (3 × Ar C), 128.62, 128.43 (4C), 128.14 (2C), 127.98 (4C), 127.87 (2C), 125.40 (2C) (15 × Ar C***H***), 108.40 (Ph***C***O_3), 73.47 (2C), 72.53 (2C) (4 × Ins ***C***H), 71.32 (2 × O***C***H_2Ph), 69.16 (Ins ***C***H), 53.09 (Ins ***C***), 49.23 (Ins 4-C***C***H_2) ppm; HRMS (ESI^+) found $[M+H]^+$ 459.1808 (17), $C_{28}H_{27}O_6$ requires 459.1808.

2-*C*-Methyl-4,6-*O*-dibenzyl-*scyllo*-inositol-1,3-*O*-benzylidine-acetal (220) and 2-*C*-methyl-1,4,6-*O*-tribenzyl-*scyllo*-inositol (221)



2-*C*-Methyl-4,6-*O*-dibenzyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**218**, 115 mg, 0.25 mmol) was evaporated from MeCN (3 × 1mL) and taken up in CH_2Cl_2 (0.6 mL). DIBAL-H (0.8 M solution in hexanes, 0.9mL, 0.75mmol) was added and the reaction stirred at rt for 3h. Excess

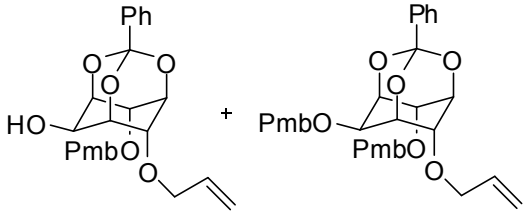
DIBAL-H was quenched by drop-wise addition of H_2O (5 mL) and the product taken up in ether, washed with 0.1% HCl, sat. $NaHCO_3$, H_2O and then brine, dried ($MgSO_4$) and all solvents removed under reduced pressure. The crude material was purified by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 7:3 v/v) afforded 2-*C*-methyl-4,6-*O*-dibenzyl-*scyllo*-inositol 1,3-*O*-benzylidine-acetal (**220**, 25 mg, 22 %), 2-*C*-methyl-1,4,6-*O*-tribenzyl-*scyllo*-inositol (**221**, 15 mg, 13 %) and starting material (**218**, 49 mg, 43 %).

2-*C*-Methyl-4,6-*O*-dibenzyl-*scyllo*-inositol-1,3-*O*-benzylidene-acetal, **220**: R_f (EtOAc-hexane, 3:7 v/v) 0.52; δ_H (400 MHz, $CDCl_3$) 7.49-7.47 (2H, m), 7.45-7.31 (13H, m) (15 × Ar-C**H**), 5.51 (1H, s, PhC***H***O_2), 4.83 (2H, d, *J* 11.6), 4.68 (2H, d, *J* 11.6) (2 × OC***H*****$_2$**Ph), 4.58 (1H, t, *J* 6.8, Ins 5-***H***), 4.05 (2H, s, 2 × Ins ***H***), 3.93 (2H, d, *J* 7.2, 2 × Ins ***H***), 1.72 (3H, s, Ins 4-CC***H*****$_3$**) ppm; δ_C (100 MHz, $CDCl_3$) 137.84 (2C), 137.30 (3 × Ar ***C***), 129.40, 128.57 (4C), 128.52 (2C), 128.09 (4C), 127.98 (2C), 126.36 (2C) (15 × Ar ***C***H), 92.52 (Ph***C***HO_2), 83.13 (2C), 79.49 (2C), 75.31 (5 × Ins ***C***H), 72.00 (2 × O***C***H_2Ph), 69.02 (Ins ***C***), 25.06 (2-C***C***H_3) ppm; HRMS (ESI^+) found $[M+H]^+$ 463.2113 (27), $C_{28}H_{31}O_6$ requires 463.2121.

2-*C*-Methyl-1,4,6-*O*-Tribenzyl-*scyllo*-inositol, **221**: R_f (EtOAc-hexane, 3:7 v/v) 0.24; δ_H (400 MHz, $CDCl_3$) 7.39-7.31 (15H, m, 15 × Ar ***H***), 4.93 (1H, d, *J* 11.2), 4.92 (1H, d, *J* 11.2), 4.91 (1H, d, *J* 11.2), 4.87 (1H, d, *J* 11.2), 4.83 (1H, d, *J* 11.2), 4.77 (1H, d, *J* 11.2) (6 × OCH***H***Ph), 3.67 (1H, t, *J* 8.8, Ins ***H***), 3.52 (1H, d, *J*10.3, Ins ***H***), 3.46 (1H, d, *J* 9.8, Ins ***H***), 3.40 (1H, t, *J* 8.8, Ins ***H***), 3.30 (1H, t, *J* 3.30, Ins ***H***), 2.19 (3H, s, CC***H*****$_3$**) ppm; δ_C (100 MHz, $CDCl_3$) 138.75, 138.54, 138.49 (3 × Ar ***C***), 128.57 (3C), 128.48 (2C), 127.98 (2C), 127.95 (3C), 127.91, 127.86, 127.78 (2C), 127.73 (15 × Ar ***C***H), 84.59, 82.69, 81.46, 75.87 (4 × Ins ***C***H), 75.55 (2 × O***C***H_2Ph), 75.41 (Ins ***C***), 75.31 (Ins ***C***H), 74.95 (O***C***H_2Ph), 21.06 (2-C***C***H_3) ppm.

2-*C*-Methyl-4,6-*O*-dibenzyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate, **218** as characterised previously.

4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (222) and 4-*O*-allyl-2,6-*O*-di(*para*-methoxybenzyl)-*myo*-inositol 1,3,5-*O*-orthobenzoate (224)



4-*O*-Allyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**92**, 1.0 g, 3.26 mmol) was evaporated from MeCN (3 × 10 mL) and taken up in DMF (10 mL). The solution was cooled to 0 ºC and NaH (60 % dispersion in mineral oil, 125 mg, 3.26 mmol) added portion-wise. After 30 min the solution was warmed to rt and *para*-methoxybenzylchloride (442 µL, 3.26 mmol) in DMF (5 mL) added

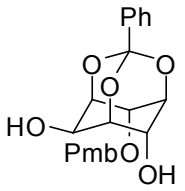
dropwise. After 6 h the reaction was quenched with H_2O and then taken up in EtOAc. The organic layer was washed with H_2O (×4) and then brine, dried ($MgSO_4$) and all solvents evaporated. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 1:5 v/v) afforded 4-*O*-allyl-6-*O*-*para*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**222**, 467 mg, 35 %) and 4-*O*-allyl-2,6-*O*-di-*para*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**224**, 171 mg, 10 %) and starting material (**92**, 110 mg, 11 %).

4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **222**: R_f (EtOAc-hexane, 1:1 v/v) 0.61; δ_H (400 MHz, $CDCl_3$) 7.66-7.64 (2H, m), 7.40-7.36 (3H, m), 7.30 (2H, d, *J* 8.8), 6.91 (2H, d, *J* 8.8) (9 × Ar ***H***), 5.93 (1H, ddt, *J* 15.7, 10.3, 5.4, $OCH_2\boldsymbol{C}CH_2$), 5.33 (1H, dq, *J* 17.1, 1.5), 5.24 (1H, dq, *J* 10.7, 1.5) (2 × $OCH_2HCH\boldsymbol{H}$), 4.67 (1H, d, *J* 11.2), 4.58 (1H, d, *J* 11.2) (2 × $OCH\boldsymbol{H}Ph$), 4.54-4.52 (1H, m, Ins ***H***), 4.47-4.43 (3H, m, 3 × Ins ***H***), 4.40-4.38 (1H, m, Ins ***H***), 4.23 (1H, bs, Ins O***H***), 4.21-4.12 (3H, m, Ins ***H*** + $OC\boldsymbol{H_2}CHCH_2$), 3.84 ($OC\boldsymbol{H}_3$) ppm; δ_H (400 MHz, d_6-DMSO) 7.55-7.53 (2H, m), 7.37-7.34 (3H, m), 7.29 (2H, d, *J* 8.3), 6.90 (2H, d, *J* 8.8) (9 × Ar ***H***), 5.89 (1H, ddt, *J* 17.1, 10.3, 5.4, $OCH_2C\boldsymbol{H}CH_2$), 5.40 (1H, d, *J* 6.4, Ins O***H***), 5.28 (1H, dq, *J* 17.1, 2.0), 5.16 (1H, dq, *J* 10.3, 1.5) (2 × $OCH_2HCH\boldsymbol{H}$), 4.66-4.64 (1H, m, Ins ***H***), 4.61 (1H, d, *J* 11.2), 4.52 (1H, d, *J* 11.2) (2 × $OCH\boldsymbol{H}Ph$), 4.30-4.26 (3H, m, 3 × Ins ***H***), 4.22-4.20 (1H, m, Ins ***H***), 4.19-4.13 (1H, ddt, *J* 13.2, 5.4, 1.5), 4.14-4.09 (1H, ddt, *J* 12.7, 5.4, 1.5) (2 × $OCH\boldsymbol{H}CHCH_2$), 4.00 (1H, dt, *J* 6.4, 2.4, Ins 2-***H***), 3.75 (3H, s, $OC\boldsymbol{H_3}$) ppm; δ_C (100 MHz, $CDCl_3$) 159.42, 136.94 (2 × Ar ***C***), 134.12 ($OCH_2\boldsymbol{C}HCH_2$), 129.71 (Ar ***C***), 129.65, 129.29 (2C), 128.11 (2C), 125.21 (2C) (7 × Ar ***C***H), 117.74 ($OCH_2CH\boldsymbol{C}H_2$), 113.89 (2 × Ar ***C***H), 107.95 ($Ph\boldsymbol{C}O_3$), 74.43, 74.36, 73.55, 73.14 (4 × Ins ***C***H), 71.31, 70.89 (2 × $O\boldsymbol{C}H_2PG$), 68.69, 60.68 (2 × Ins ***C***H), 55.31 ($OC\boldsymbol{H}_3$) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 427 (100); HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 427.1759, $C_{24}H_{27}O_7$ requires 427.1757.

4-*O*-Allyl-2,6-*O*-di-*para*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **224**: R_f (EtOAc-hexane, 1:1 v/v) 0.53; δ_H (400 MHz, $CDCl_3$) 7.68-7.66 (2H, m), 7.37-7.34 (5H, m), 7.23 (2H, d, *J* 8.3), 6.89 (2H, d, *J* 8.8), 6.88 (2H, d, *J* 8.8) (13 × Ar ***H***), 5.87 (1H, ddt, *J* 16.1, 10.7, 5.9, $OCH_2C\boldsymbol{H}CH_2$), 5.26 (1H, dq, *J* 17.1, 1.5), 5.20 (1H, dq, *J* 10.3, 1.5) (2 × $OCH_2HCH\boldsymbol{H}$), 4.67 (2H, s, $OC\boldsymbol{H_2}Ph$), 4.60 (1H, d, *J* 11.7, $OCH\boldsymbol{H}Ph$), 4.52-4.49 (2H, m, 2 × Ins ***H***), 4.46 (1H, d, *J* 11.2, $OCH\boldsymbol{H}Ph$), 4.43-4.39 (3H, m, 3 × Ins ***H***), 4.12 (1H, ddt, *J* 12.7, 5.4, 1.5, $OCH\boldsymbol{H}CHCH_2$), 4.05 (1H, m, Ins ***H***), 4.03 (1H, ddt, *J* 13.2, 5.9, 1.5, $OCH\boldsymbol{H}CHCH_2$) 3.84 (3H, s, $OC\boldsymbol{H_3}$), 3.81 (3H, m, $OC\boldsymbol{H_3}$) ppm; δ_C (100 MHz, $CDCl_3$) 159.35, 159.06 (2 × Ar ***C***), 134.22 ($OCH_2\boldsymbol{C}HCH_2$), 130.27, 130.23, 129.89 (3 × Ar ***C***), 129.62 (2C), 129.37, 129.19 (2C), 127.93 (2C), 125.41 (2C) (9 × Ar ***C***H), 117.49 ($OCH_2CH\boldsymbol{C}H_2$), 113.82 (4 × Ar ***C***H), 106.66 ($Ph\boldsymbol{C}O_3$), 73.87, 73.62, 72.07 (2C) (4 × Ins ***C***H), 71.19, 70.93, 70.68 (3 × $O\boldsymbol{C}H_2PG$), 69.12, 65.95 (2 × Ins ***C***H), 55.31, 55.27 (2 × $O\boldsymbol{C}H_3$) ppm; MS (CI^+) *m/z* (%) found $[M+NH_4]^+$ 547 (7).

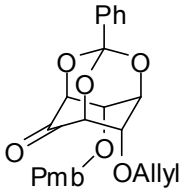
4-*O*-Allyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **92**: Data as previously reported.

6-*O*-*para*-Methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (223)



To a solution of 4-*O*-allyl-6-*O*-*para*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**222**, 120 mg, 0.29 mmol) in dioxane (1 mL) and H_2O (0.1 mL) was added a mixture of 4-methyl morpholine *N*-oxide (118 mg, 0.88 mmol) and OsO_4 (4 % solution, 3 drops). After 1 h, $NaIO_4$ (188 mg, 0.88 mmol) was added and the solution stirred for a further 12 h. The solution was diluted with brine and the product extracted into CH_2Cl_2. The organic layer was washed with H_2O and then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 3:2 v/v) afforded the *title compound* (**223**, 50 mg, 44 %) as a pale brown residue; R_f (EtOAc-hexane, 3:2 v/v) 0.43; δ_H (400 MHz, $CDCl_3$) 7.64-7.62 (2H, m), 7.39-7.36 (3H, m), 7.27 (2H, d, *J* 8.8), 6.93 (2H, d, *J* 8.8) (9 × Ar ***H***), 4.66 (1H, d, *J* 11.7), 4.62 (1H, d, *J* 11.2) (2 × OCH***H***Ph), 4.61-4.56 (1H, m, Ins ***H***), 4.53 (1H, td, *J* 3.4, 1.5, Ins ***H***), 4.43-4.39 (2H, m, 2 × Ins ***H***), 4.37 (1H, td, *J* 3.4, 1.5, Ins ***H***), 4.17-4.13 (1H, m, Ins ***H***), 3.83 (3H, s, OC***H₃***), 2.94 (1H, s, Ins O***H***), 2.88 (1H, s, Ins O***H***) ppm; δ_C (100 MHz, $CDCl_3$) 160.04, 136.64 (2 × Ar ***C***), 129.93 (2C), 129.72, 128.10 (2C) (5 × Ar ***C***H), 127.97 (Ar ***C***), 125.25 (2C), 114.30 (2C) (4 × Ar ***C***H), 107.41 (Ph***C***O_3), 76.09, 73.67, 73.64 (3 × Ins ***C***H), 72.81 (O***C***H_2Ph), 68.23, 67.79, 59.98 (3 × Ins ***C***H), 55.34 (O***C***H_3) ppm; HRMS (ESI^+) found $[M+H]^+$ 387.1438 (5), $C_{21}H_{23}O_7$ requires 387.1444.

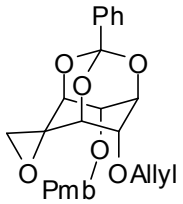
4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-inos-2-ose 1,3,5-*O*-orthobenzoate (225)



4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**222**, 1.00 g, 2.44 mmol) was evaporated from MeCN (3 × 5 mL) and taken up in CH_2Cl_2 (10 mL). DMP (2.07 g, 4.88 mmol) was added portion-wise and the reaction stirred at rt for 3 h. The reaction was quenched by stirring with sat. $NaHCO_3$ and $Na_2O_3S_2.H_2O$ for 30 min. The product was taken up in CH_2Cl_2 and the organic layer washed with sat. $NaHCO_3$, H_2O and then brine, dried ($MgSO_4$), and all solvents removed under reduced pressure. 4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-inos-2-ose 1,3,5-*O*-orthobenzoate (**225**, 9.95 g, 100 %) was recovered as a pale yellow oil and used without further purification: R_f (EtOAc-hexane, 2:3 v/v) 0.39; δ_H (400 MHz, $CDCl_3$) 7.67-7.64 (2H, m), 7.41-7.38 (3H, m), 7.28 (2H, d, *J* 8.8), 6.90 (2H, d, *J* 8.3) (9 × Ar ***H***), 5.89 (1H, ddt, 17.1, 10.3, 5.9,

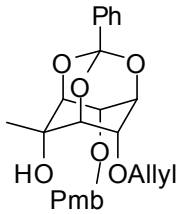
$OCH_2C\boldsymbol{H}CH_2$), 5.30 (1H, dq, *J* 17.6, 1.5), 5.24 (1H, dq, *J* 10.3, 1.5) (2 × $OCH_2HCH\boldsymbol{H}$), 4.70-4.67 (3H, m, 3 × Ins ***H***), 4.61 (2H, s, $OC\boldsymbol{H_2}Ph$), 4.58-4.55 (1H, m, Ins ***H***), 4.52-4.50 (1H, m, Ins ***H***), 4.15 (2H, dt, *J* 5.9, 1.5, $OC\boldsymbol{H_2}CHCH_2$), 3.83 (3H, s, $OC\boldsymbol{H_3}$) ppm; δ_C (100 MHz, $CDCl_3$) 200.09 (***C***=O), 159.52, 135.27 (2 × Ar ***C***), 133.69 ($OCH_2\boldsymbol{C}HCH_2$), 129.66, 129.50 (2C) (3 × Ar ***C***H), 129.03 (Ar ***C***), 128.16 (2C), 125.50 (2C) (4 × Ar ***C***H), 118.28 ($OCH_2CH\boldsymbol{C}H_2$), 113.93 (2 × Ar ***C***H), 108.12 ($Ph\boldsymbol{C}O_3$), 78.51, 76.70, 76.12, 74.27 (4 × Ins ***C***H), 71.23, 70.88 (2 × $O\boldsymbol{C}H_2PG$), 69.77 (Ins ***C***H), 55.31 ($O\boldsymbol{C}H_3$) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 425 (100 %); HRMS (CI^+) *m/z* (%) found $[M+H]^+$ 425.1599 (100), $C_{24}H_{25}O_7$ requires 425.1600.

4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-2,2-*O*,*C*-methylidene-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (226)



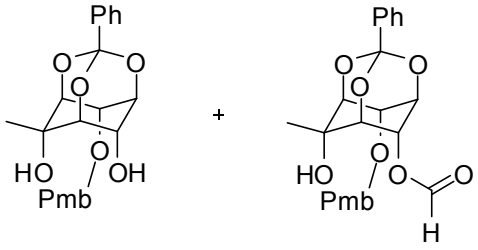
To a clear solution of trimethylsulfoxonium iodide (1.18 g, 5.36 mmol) in DMSO (10 mL) was added NaH (60% dispersion in mineral oil, 224 mg, 5.85 mmol) and the mixture stirred for 40 min. 4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-inos-2-ose 1,3,5-*O*-orthobenzoate (**225**, 2.07 g, 4.88 mmol) was evaporated from MeCN (3 × 10 mL), taken up in THF (10 mL) and added to the reaction mixture, which was stirred at rt for 1 h. The reaction was quenched by drop-wise addition of H_2O (10 mL) and taken up in CH_2Cl_2. The organic layer was washed with sat. $NaHCO_3$, H_2O, then brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 2:3 v/v) afforded the *title compound* (**226**, 1.45 g, 68 %) as a clear oil; R_f (EtOAc-hexane, 2:3 v/v) 0.48; δ_H (400 MHz, $CDCl_3$) 7.66-7.64 (2H, m), 7.39-7.35 (5H, m), 6.89 (2H, d, *J* 8.8) (9 × Ar ***H***), 5.96 (1H, ddt, *J* 17.1, 10.3, 5.4, $OCH_2C\boldsymbol{H}CH_2$), 5.33 (1H, dq, *J* 17.1, 1.5), 5.23 (1H, dq, *J* 10.3, 1.5) (2 × $OCH_2HCH\boldsymbol{H}$), 4.68 (1H, tt, *J* 3.4, 2.0, Ins 5-***H***), 4.66 (2H, s, $OC\boldsymbol{H_2}Ph$), 4.46-4.43 (2H, m, Ins 4-***H*** + Ins 6-***H***), 4.18 (2H, ddt, *J* 6.9, 1.5, 1.0, $O\boldsymbol{C}H_2CHCH_2$), 3.99 (1H, dt, *J* 3.4, 1.5, Ins 1/3-***H***), 3.95 (1H, dt, *J* 3.4, 2.0, Ins 1/3-***H***), 3.83 (3H, s, $OC\boldsymbol{H_3}$) ppm; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 439.1748 (7), $C_{25}H_{27}O_7$ requires 439.1757.

4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (227)



4-*O*-Allyl-6-*O*-*para*-methoxybenzyl-2,2-*O*,*C*-methylidene-*scyllo*-inositol 1,3,5-*O*-ortho benzoate (**226**, 1.45 g, 3.31 mmol) was evaporated from MeCN (3 × 5 mL) and taken up in THF (15 mL). $LiAlH_4$ (251 mg, 6.62 mmol) was added and the resulting suspension was stirred for 3 h. The reaction was quenched by drop-wise addition of EtOAc and the crude material was taken up in ether. The organic layer was washed with sat. $NaHCO_3$, H_2O then brine, dried ($MgSO_4$) and all solvents removed under reduced pressure. The crude material was purified by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 3:7 v/v) afforded the *title compound* (**227**, 1.28 g, 88 %) as a yellow oil: R_f (EtOAc-hexane, 7:13 v/v) 0.66; δ_H (400 MHz, $CDCl_3$) 7.65-7.62 (2H, m), 7.40-7.38 (3H, m), 7.30 (2H, d, *J* 8.8), 6.91 (2H, d, *J* 8.3) (9 × Ar ***H***), 5.92 (1H, ddt, *J* 16.1, 10.6, 5.9, $OCH_2C\boldsymbol{H}CH_2$), 5.29 (1H, dq, *J* 17.1, 1.5), 5.23 (1H, dq, *J* 10.8, 1.5) (2 × $OCH_2HCH\boldsymbol{H}$), 4.72 (1H, bs, Ins 2-O***H***), 4.69 (1H, d, *J* 11.2, OCH***H***Ph), 4.69-4.67 (1H, m, Ins ***H***), 4.66 (1H, d, *J* 11.2, OCH***H***Ph), 4.55-4.53 (2H, m, 2 × Ins ***H***), 4.25-4.21 [3H, m, (2 × Ins ***H***) + $OCH\boldsymbol{H}CHCH_2$], 4.18 (1H, ddt, *J* 12.2, 5.9, 1.5, $OCH\boldsymbol{H}CHCH_2$) 3.84 (3H, s, $OC\boldsymbol{H_3}$), 1.66 (3H, s, 2-$CC\boldsymbol{H_3}$) ppm; δ_C (100 MHz, $CDCl_3$) 159.43, 136.55 (2 × Ar ***C***), 133.52 ($OCH_2\boldsymbol{C}HCH_2$), 129.45 (3 × Ar ***C***H), 129.01 (Ar ***C***), 128.03 (2C), 125.23 (2C) (4 × Ar ***C***H), 117.81 ($OCH_2CH\boldsymbol{C}H_2$), 113.83 (2 × Ar ***C***), 107.39 (Ph$\boldsymbol{C}O_3$), 74.11, 74.08, 73.87, 73.47 (4 × Ins ***C***H), 71.25, 70.54 (2 × O$\boldsymbol{C}H_2$PG), 68.41 (Ins ***C***H), 67.66 (Ins ***C***), 55.23 (O$\boldsymbol{C}H_3$), 24.89 (2-C$\boldsymbol{C}H_3$) ppm; HRMS (ESI^+) *m/z* (%) found $[M+H]^+$ 441.1893 (100), $C_{25}H_{29}O_7$ requires 441.1913.

6-*O*-*para*-Methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (228) and 4-*O*-formyl-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (229)



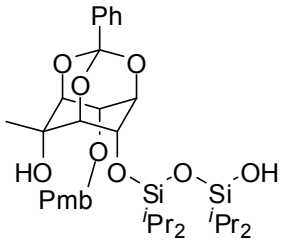
To a solution of 4-*O*-allyl-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**227**, 360 mg, 0.85 mmol) in dioxane (3 mL) and H_2O (0.3 mL) was added a mixture of 4-methyl morpholine *N*-oxide (344 mg, 2.55 mmol) and OsO_4 (4 % solution, 10 drops). After 1 h, $NaIO_4$ (545 mg, 2.55 mmol) was added and the solution stirred for a further 12 h. The solution was diluted with brine and the product extracted into CH_2Cl_2. The organic layer was washed with H_2O and then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (0:1 → 3:2 v/v) afforded 6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**228**, 220 mg, 65 %) as a glass and 4-*O*-formyl-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**229**, 74 mg, 20 %) as a pale yellow oil.

6-*O*-*para*-Methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate **228**; R_f (EtOAc-hexane, 3:7 v/v) 0.29; δ_H (500 MHz, $CDCl_3$) 7.61-7.59 (2H, m), 7.37-7.34 (3H, m), 7.29 (2H, d, *J* 8.3), 6.89 (2H, d, *J* 8.3) (9 × Ar ***H***), 4.71 (1H, d, *J* 11.2, OCH***H***Ph), 4.69-4.67 (1H, m, Ins ***H***), 4.66 (1H, d, *J* 11.2, OCH***H***Ph), 4.56-4.54 (2H, m, 2 × Ins ***H***), 4.18 (1H, td, *J* 2.9, 2.5, Ins 1/3-***H***), 4.12 (1H, dt, *J* 2.0, 3.9, Ins 4/6-***H***), 4.11 (1H, bs, Ins O***H***), 3.54 (1H, bs, Ins O***H***), 3.80 (3H, s, OC***H₃***), 1.64 (3H, s, 2-CC***H₃***) ppm; δ_C (125 MHz, $CDCl_3$) 159.73, 136.39 (2 × Ar ***C***), 129.83 (2C), 129.54 (3 × Ar ***C***H), 128.54 (Ar ***C***), 128.17 (2C), 125.28 (2C), 114.09 (2C) (6 × Ar ***C***), 107.12 (Ph***C***O_3), 75.40, 73.89 (2C) (3 × Ins ***C***H), 71.88 (O***C***H_2Ar), 69.74 (Ins ***C***H), 68.68 (Ins ***C***), 68.22 (Ins ***C***H), 55.27 (O***C***H_3), 25.91 (2-C***C***H_3) ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 401 (100 %); HRMS (CI^+) *m/z* (%) found $[M+H]^+$ 401.1604 (100), $C_{22}H_{25}O_7$ requires 401.1604.

4-*O*-Formyl-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate **229**: R_f (EtOAc-hexane, 3:7 v/v) 0.48; δ_H (500 MHz, $CDCl_3$) 8.04 (1H, d, *J* 1.0, 4-OCO***H***), 7.62-7.59 (2H, m), 7.39-7.35 (3H, m), 7.26 (2H, d, *J* 8.8), 6.90 (2H, d, *J* 8.8) (9 × Ar ***H***), 5.81 (1H, t, *J* 3.4, Ins 4-***H***), 4.69 (1H, tt, *J* 3.4, 1.5, Ins 5-***H***), 4.67 (1H, d, *J* 11.2), 4.64 (1H, d, *J* 11.2) (2 × OCH***H***Ph), 4.55 (1H, td, *J* 3.4, 1.5, Ins 6-***H***), 4.49 (1H, d, *J* 1.5, Ins 2-O***H***), 4.24 (1H, dt, *J* 3.9, 2.0, Ins 1/3-***H***), 4.19 (1H, dt, *J* 3.4, 1.5, Ins 1/3-***H***), 3.84 (3H, s, OC***H₃***), 1.65 (3H, s, 2-CC***H₃***) ppm; δ_C (125 MHz, $CDCl_3$) 159.77 (Ar ***C***), 159.39 (4-O***C***OH), 136.01 (Ar ***C***), 129.65, 129.60 (2C) (3 × Ar ***C***H), 128.15 (Ar ***C***), 128.06 (2C), 125.27 (2C), 114.08 (2C) (6 × Ar ***C***H), 107.47 (Ph***C***O_3), 74.03, 73.41, 73.01 (3 × Ins ***C***H), 72.02 (O***C***H_2Ar), 68.13 (Ins ***C***H), 67.54 (Ins ***C***), 67.38 (Ins ***C***H), 55.25 (O***C***H_3), 24.84 (2-C***C***H_3) ppm.

4-*O*-Formyl-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**229**, 74 mg, 0.17 mmol) was converted to 6-*O*-*para*-Methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**228**, 67 mg, 97 %) by stirring with Et_3N-EtOH (1:2) for 1 h.

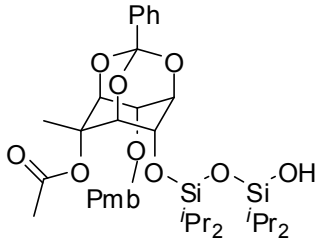
4-*O*-(1,1,3,3-Tetra-*iso*-propyl-1,3-disiloxane)-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate, 230



6-*O*-*para*-Methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**228**, 430 mg, 1.07 mmol) and imidazole (440 mg, 6.45 mmol) were evaporated from MeCN (3 × 3 mL) and taken up in DMF (10 mL). 1,3-dichloro-1,1,3,3-tetra-*iso*-propyl-1,3-disiloxane (506 mg, 1.60 mmol) was added drop-wise and the solution stirred for 18 h. The reaction was quenched with H_2O (0.5 mL) and triethylamine (1 mL). After 30 min the volume was reduced under vacuum and the

residue taken up in EtOAc. The organic layer was washed with sat. $NaHCO_3$, H_2O and then brine, dried ($MgSO_4$) and all solvents removed under reduced pressure. The crude material (722 mg) was fractionated by chromatography on flash silica. Elution with EtOAc-hexane (1:9 → 3:7 v/v) afforded the *title compound* (**230**, 322 mg, 65 %) as a pale yellow oil; R_f (EtOAc-hexane, 3:7 v/v) 0.62; δ_H (400 MHz, $CDCl_3$) 7.64-7.62 (2H, m), 7.39-7.37 (3H, m), 7.31 (2H, d, *J* 8.3), 6.90 (2H, d, *J* 8.8) (9 × Ar ***H***), 4.93 (1H, td, *J* 3.4, 2.0, Ins 4-***H***), 4.90 (1H, bs, SiO***H***), 4.71 (1H, d, *J* 11.2, OCH***H***Ph), 4.63 (1H, d, *J* 11.2, OCH***H***Ph), 4.55-4.54 (2H, m, 2 × Ins ***H***), 4.24-4.21 (2H, m, 2 × Ins ***H***), 3.83 (3H, s, OC***H₃***), 3.76 (1H, bs, Ins 2-O***H***), 1.68 (3H, s, 2-CC***H₃***), 1.34-1.28 [4H, m, 4 × SiC***H***$(CH_3)_2$], 1.10-1.01 [24H, m, 4 × SiCH(C***H₃***$)_2$] ppm; δ_C (100 MHz, $CDCl_3$) 159.68, 136.58 (2 × Ar ***C***), 129.99 (2C), 129.53 (3 × Ar ***C***H), 128.54 (Ar ***C***), 128.09 (2C), 125.35 (2C), 113.94 (2C) (6 × Ins ***C***H), 107.23 (Ph***C***O_3), 75.94, 73.51, 73.48 (3 × Ins ***C***H), 71.57 (O***C***H_2Ar), 70.68, 68.47 (2 × Ins ***C***H), 68.27 (Ins ***C***), 55.29 (O***C***H_3), 24.92 (2-C***C***H_3), 17.36 (2C), 17.27 (2C), 17.19, 17.08, 17.03, 14.14, 13.58, 13.55, 12.90, 12.33 [4 × Si***C***H(CH_3) + 6 × SiCH(***C***H_3)] ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 661 (44 %); HRMS (CI^+) *m/z* (%) found $[M+H]^+$ 661.3211 (100), $C_{34}H_{53}O_9Si_2$ requires 661.3228.

4-*O*-(1,1,3,3-Tetra-*iso*-propyl-1,3-disiloxane)-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-2-*O*-acetyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (231)



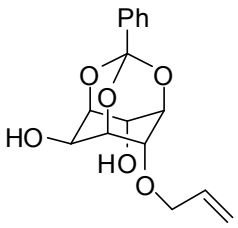
4-*O*-(1,1,3,3-Tetra-*iso*-propyl-1,3-disiloxane)-6-*O*-*para*-methoxybenzyl-2-*C*-methyl-*scyllo*-inositol 1,3,5-*O*-orthobenzoate (**230**, 33 mg, 0.05 mmol) was taken up in CH_2Cl_2 (0.5 mL). DMAP (1 mg, 0.01 mmol), acetic anhydride (29 µL, 0.31 mmol) and triethylamine (72 µL, 0.51 mmol) were added and the solution stirred for 2 h. The reaction was quenched by addition of H_2O, and taken up in CH_2Cl_2. The organic layer was washed with H_2O, then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure to afford the *title compound* (**231**, 35 mg, 100 %) as a clear oil; R_f (EtOAc-hexane, 3:7 v/v) 0.73; δ_H (400 MHz, $CDCl_3$) 7.61-7.59 (2H, m), 7.37-7.35 (3H, m), 7.29 (2H, d, *J* 8.8), 6.88 (2H, d, *J* 8.8) (9 × Ar ***H***), 4.98 (1H, td, *J* 3.4, 1.5, Ins 4-***H***), 4.70 (1H, d, *J* 10.7, OCH***H***Ph), 4.65 (1H, d, *J* 1.5, SiO***H***), 4.59 (1H, d, *J* 11.2, OCH***H***Ph), 4.54-4.52 (2H, m, 2 × Ins ***H***), 4.24 (1H, td, *J* 4.9, 2.0, Ins 6-***H***), 4.13-4.11 (1H, m, Ins ***H***), 3.83 (3H, s, OC***H₃***), 2.10 (3H, s, OCOC***H₃***), 1.64 (3H, s, 2-CC***H₃***), 1.29-1.23 [4H, m, 4 × SiC***H***$(CH_3)_2$], 1.12-1.00 [24H, m, 6 × SiCH(C***H₃***$)_2$] ppm; δ_C (100 MHz, $CDCl_3$) 170.65 (O***C***OCH_3), 159.52, 136.08 (2 × Ar ***C***), 129.84 (2C), 129.43 (3 × Ar ***C***H), 129.07 (Ar ***C***), 128.05 (2C), 125.32 (2C), 113.83 (2C) (6 × Ar ***C***H), 107.21 (Ph***C***O_3), 76.11, 73.84, 73.54 (3 × Ins ***C***H), 71.38 (O***C***H_2Ar), 70.81, 68.07 (2 × Ins

CH), 67.88 (Ins ***C***), 55.32 (O***C***H_3), 25.17 (2-C***C***H_3), 22.55 (OCO***C***H_3), 17.05, 17.02 (2C), 16.95 (5C), 13.06 (2C), 12.93, 12.86 [4 × Si***C***H(CH_3) + 6 × SiCH(***C***H_3)] ppm; MS (CI^+) *m/z* (%) found $[M+H]^+$ 703 (15), $[M+Na]^+$ 725 (100); HRMS (CI^+) *m/z* (%) found $[M+Na]^+$ 703.3334, $C_{36}H_{55}O_{10}Si_2$ requires 703.3334.

Appendix A: Preparation of 2,6-*O*-dibenzyl-inos-4-ose 1,3,5-*O*-orthobenzoate

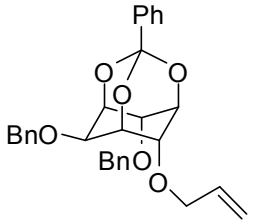
The development and optimisation of these experimental proceedures was carried out during my MChem project, October 2004-June 2005.

4-*O*-Allyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (92).



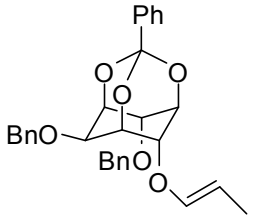
myo-Inositol (**1**, 5.00 g, 27 mmol) was taken up in DMSO (23 mL). To the suspension was added trimethylorthobenzoate (6.01 mL, 33.3 mmol) and *p*-toluene sulfonic acid (56 mg, 0.29 mmol). The suspension was stirred at 100 ºC for 5 h. The reaction was quenched with methylamine (0.9 mL) and the solvent removed under high vacuum. Crude *myo*-inositol 1,3,5-*O*-orthobenzoate (**22d**, 7.6 g) was taken up in DMF (50 mL). The reaction mixture was cooled to -15 ºC and sodium hydride (60 % dispersion in mineral oil, 1.08 g, 27 mmol) added portion wise. The mixture was stirred at -15 ºC for 15 min, allowed to warm to rt and stirred for a further 30 min. Allyl bromide (2.34 mL, 27 mmol) was then added drop-wise and the reaction stirred for 24 h. The reaction was quenched by drop-wise addition of H_2O (5 mL) and the volume reduced under high vacuum. The residue was taken up in EtOAc and washed with H_2O (× 3) and then brine. The combined aqueous layers were back-extracted with EtOAc, and this solution was washed with H_2O (× 3) and brine. The organic layers were combined, dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (7:3 → 0:1 v/v) yielded the *title compound* (**92**, 6.03 g, 73 % over 2 steps); R_f (EtOAc) 0.72; δ_H (400 MHz, $CDCl_3$) 7.66-7.60 (2H, m, Ar ***H***), 7.42-7.26 (3H, m, Ar ***H***), 5.90 (1H, ddt, *J* 16.3, 10.3, 5.9, $OCH_2C\boldsymbol{H}CH_2$), 5.36 (1H, dq, *J* 15.7, 1.2, $OCH_2CHC\boldsymbol{H}_aH$), 5.32 (1H, dq, *J* 10.3, 1.2, $OCH_2CHC\boldsymbol{H}_bH$), 4.61 (1H, dtd**,** *J* 10.0, 4.1, 1.2, Ins 6-***H***), 4.52-4.47 (2H, m, 2 × Ins ***H***), 4.45-4.39 (2H, m, 2 × Ins ***H***), 4.21-4.16 (3H, m, Ins ***H*** + $OC\boldsymbol{H}_2CHCH_2$), 3.73 (1H, bd**,** *J* 10.3, Ins O***H***), 3.19 (1H, bd, *J* 11.8, Ins O***H***) ppm; δ_C (100 MHz, $CDCl_3$) 136.52 (Ar ***C***), 132.64 ($OCH_2\boldsymbol{C}HCH_2$), 129.70, 128.07 (2C), 125.17 (2C) (5 × Ar ***C***H), 119.56 ($OCH_2CH\boldsymbol{C}H_2$), 107.37 ($Ph\boldsymbol{C}O_3$), 76.02, 74.04, 73.53 (3 × Ins ***C***H), 71.99 ($O\boldsymbol{C}H_2CHCH_2$), 68.16, 67.69, 59.93 (3 × Ins ***C***H) ppm; HRMS (CI^+) *m/z* (%) found $[M+H]^+$ 307.1189 (100), $C_{16}H_{19}O_6$ requires 307.1182.

2,6-*O*-Dibenzyl-4-*O*-allyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (93).



4-*O*-Allyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**92**, 11.30 g, 36.9 mmol) was evaporated from MeCN (3 × 10 mL), taken up in DMF (100 mL) and cooled to -15 °C. Sodium hydride (60 % dispersion in mineral oil, 4.25 g, 111 mmol) was added portion-wise, the reaction stirred at -15 °C for 30 min then warmed to rt and benzyl bromide (13.2 mL, 111 mmol) was added drop-wise. The reaction was stirred at 60 °C overnight then quenched by drop-wise addition of H_2O (5 mL) and stirred for 30 min. The solvent volume was reduced under high vacuum, the residue dissolved in EtOAc and washed with H_2O (× 3) then brine. The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica in a large sinter funnel. Elution with hexane-EtOAc (9:1 → 7:3 v/v) afforded the *title compound* (**93**, 17.30 g, 97 %) as a yellow oil; R_f(hexane-EtOAc 1:1 v/v) 0.76; δ_H (400 MHz, $CDCl_3$) 7.66-7.22 (15H, m, Ar ***H***), 5.82 (1H, ddt, *J* 17.1, 10.7, 5.6, $OCH_2C\boldsymbol{H}CH_2$), 5.24 (1H, dq, *J* 17.2, 1.5, $OCH_2CHC\boldsymbol{H}H$), 5.18 (1H, dq, *J* 10.5, 1.2, $OCH_2CHC\boldsymbol{H}H$), 4.70 (2H, s, $OC\boldsymbol{H}_2Ph$), 4.67 (1H, d, *J* 11.8 $OCH\boldsymbol{H}Ph$), 4.51 (1H, d, *J* 11.8, $OCH\boldsymbol{H}Ph$), 4.55-4.52 (2H, m, 2 × Ins ***H***), 4.50 (1H, dq, *J* 3.8, 1.7, Ins ***H***), 4.47 (1H, dt, *J* 3.5, 1.6, Ins ***H***), 4.43 (1H, dt, *J* 3.7, 1.6, Ins ***H***), 4.13 (1H, ddt, *J* 12.7, 5.7, 1.7, $OCH\boldsymbol{H}CHCH_2$), 4.10 (1H, t, *J* 1.4, Ins 2-***H***), 4.04 (1H, ddt, *J* 12.7, 5.7, 1.7, $OCH\boldsymbol{H}CHCH_2$) ppm; δ_C (100 MHz, $CDCl_3$) 138.13, 137.77, 137.18 (3 × Ar ***C***), 134.13 ($OCH_2\boldsymbol{C}HCH_2$), 129.39, 128.42 (4C), 128.00 (2C), 127.94 (2C), 127.84, 127.75, 127.54 (2C), 125.39 (2C) (15 × Ar ***C***H), 117.56 ($OCH_2CH\boldsymbol{C}H_2$), 107.85 ($Ph\boldsymbol{C}O_3$), 73.83, 72.06, 71.92 (3 × Ins ***C***H), 71.49, 71.29, 70.71 [(2 × $O\boldsymbol{C}H_2Ph$) + $O\boldsymbol{C}H_2CHCH_2$], 69.05, 66.34, 60.41 (3 × Ins ***C***H) ppm; HRMS (CI+) *m/z* (%) found $[M+H]^+$ 487.2130 (100), $C_{30}H_{31}O_6$ requires 487.2121.

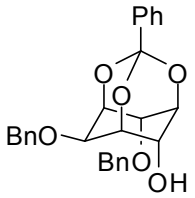
2,6-*O*-Dibenzyl-4-*O*-(prop-1-enyl)-*myo*-inositol 1,3,5-*O*-orthobenzoate (94).



2,6-*O*-Dibenzyl-4-*O*-allyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**93**, 5.40 g, 11.1 mmol) was evaporated from MeCN (3 × 5 mL), dissolved in DMSO (11 mL) and potassium *t*-butoxide (2.50 g, 22.2 mmol) added. The solution was stirred at 100 °C for 3 h. The reaction was cooled, diluted

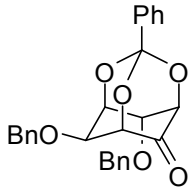
with H_2O (5 mL) and extracted with EtOAc. The organic layer was washed with H_2O (× 4), then brine, dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The *title compound* (**94**, 5.40 g, 100 %) was isolated as a yellow oil and used without further purification; R_f(hexane-EtOAc 1:1 v/v) 0.76; δ_H (400 MHz, $CDCl_3$) 7.61-7.68 (2H, m, Ar ***H***), 7.22-7.46 (13H, m, Ar ***H***), 6.03 (1H, dq, *J* 6.1, 1.7, OC***H***CHCH$_3$), 4.72 (2H, s, OC***H$_2$***Ph), 4.67-4.62 (1H, m, Ins ***H***), 4.66 (1H, d, *J* 11.8, OCH***H***Ph), 4.50 (1H, d, *J* 12.2, OCH***H***Ph), 4.57-4.42 [5H, m, (4 × Ins ***H***) + OCHC***H***CH$_3$], 4.10 (1H, t, *J* 1.6, Ins 2-***H***), 1.39 (3H, dd, *J* 6.8, 1.5, OCHCHC***H$_3$***) ppm; δ_C (100 MHz, $CDCl_3$) 143.26 (O***C***HCHCH$_3$), 137.83, 137.56, 136.93 (3 × Ar ***C***), 129.44, 128.42 (2C), 128.34 (2C), 128.04 (2C), 127.93 (2C), 127.80 (2C), 127.54 (2C), 125.36 (2C) (15 × Ar ***C***H), 107.83 (Ph***C***O$_3$), 103.96 (OCH***C***HCH$_3$), 75.17, 73.51, 71.94, 71.73 (4 × Ins ***C***H), 71.45, 71.10 (2 × O***C***H$_2$Ph), 69.06, 65.59 (2 × Ins ***C***H), 14.17 (OCHCH***C***H$_3$) ppm; HRMS (CI$^+$) *m/z* (%) found $[M+H]^+$ 487.2130 (100) $C_{30}H_{31}O_6$ requires 487.2121.

2,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (95).



2,6-*O*-Dibenzyl-4-*O*-(prop-1-enyl)-*myo*-inositol 1,3,5-*O*-orthobenzoate (**94**, 17.41 g, 35.78 mmol) was taken up in MeCN (50 mL) and H_2O (5 mL). To the vigorously stirred solution was added *p*-toluene sulfonic acid (680 mg, 3.58 mmol). After 48 h the reaction was quenched with triethylamine (1.5 mL) and concentrated under vacuum. The residue was taken up in EtOAc, then washed with sat. $NaHCO_3$, H_2O (× 2) and brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica in a large sinter funnel. Elution with hexane-EtOAc (1:0 → 1:1 v/v) afforded the *title compound* (**95**, 12.61 g, 79 %) as a colourless oil; R_f(hexane-EtOAc 4:1 v/v) 0.16; δ_H (400 MHz, $CDCl_3$) 7.66-7.19 (15H, m, 15 × Ar ***H***), 4.82 (1H, d, *J* 12.5, PhCH***H***O), 4.68 (1H, d, *J* 12.5, PhCH***H***O), 4.58-4.53 [4H, m, (2 × Ins C***H***) + PhC***H$_2$***O], 4.50-4.47 (2H, m, 2 × Ins C***H***), 4.40-4.38 (1H, m, Ins C***H***), 3.94-3.93 (1H, m, Ins C***H***), 3.63 (1H, bs, Ins O***H***) ppm; δ_C (100 MHz, $CDCl_3$) 137.81, 136.79, 135.93 (3 × Ar ***C***), 129.46, 128.84 (2C), 128.75, 128.53 (2C), 128.11 (2C), 127.93 (5C), 125.37 (2C) (15 × Ar ***C***H), 107.32 (Ph***C***O$_3$), 74.46, 73.41 (2 × Ins ***C***H), 72.96 (O***C***H$_2$Ph), 71.29 (Ins ***C***H), 70.99 (O***C***H$_2$Ph), 68.67, 67.94, 65.07 (3 × Ins ***C***H) ppm; HRMS (ESI$^+$) *m/z* (%) found $[M+H]^+$ 447.1793 (100), $C_{27}H_{27}O_6$ requires 447.1808.

2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-*O*-orthobenzoate (96).



2,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**95**, 2.00 g, 4.47 mmol) was evaporated from MeCN (3 × 5 mL) and taken up in CH_2Cl_2 (20 mL). Dess Martin periodinane (3.80 g, 4.94 mmol) was added portion-wise and the suspension stirred for 3 h. $Na_2S_2O_3.H_2O$ (10 % solution in H_2O) was added and stirring continued for 10 min. The product was extracted with CH_2Cl_2 (× 2), and the combined organic layers washed successively with sat. $NaHCO_3$, H_2O and brine, before drying ($MgSO_4$) and evaporation to dryness under reduced pressure. The crude inos-4-ose (**96**, 1.98 g, 100 %) was used without further purification; R_f (hexane-acetone, 7:3 v/v) 0.21; IR υ(C=O) 1766.5 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.68-7.63 (3H, m, Ar ***H***), 7.43-7.20 (12H, m, Ar ***H***), 4.81-4.45 [8H, m, (4 × Ins ***H***) + (2 × OC***H₂***Ph)], 3.82-3.81 (1H, m, Ins ***H***) ppm; δ_C (100 MHz, $CDCl_3$) 200.01 (Ins ***C***=O), 137.14, 136.33, 135.66 (3 × Ar ***C***), 129.80, 128.67 (2C), 128.58 (2C), 128.45, 128.11, 128.05 (2C), 127.94 (2C), 127.89 (2C), 125.49 (2C) (15 × Ar ***C***H), 108.04 (O_3***C***Ph), 79.77, 77.40, 71.95 (3 × Ins ***C***H), 71.76, 71.49 (2 × O***C***H_2Ph), 70.46, 70.38 (2 × Ins ***C***H) ppm; HRMS (CI+) *m/z* (%) found $[M+H]^+$ 445.1666 (100), $C_{27}H_{25}O_6$ requires 445.1651.

Appendix B: Crystal Structure Data

For conduritol derivative 6-benzyloxy-2-*iso*butyl-2-*iso*propyl-cyclohex-4-ene-1,2,3-triol, 138

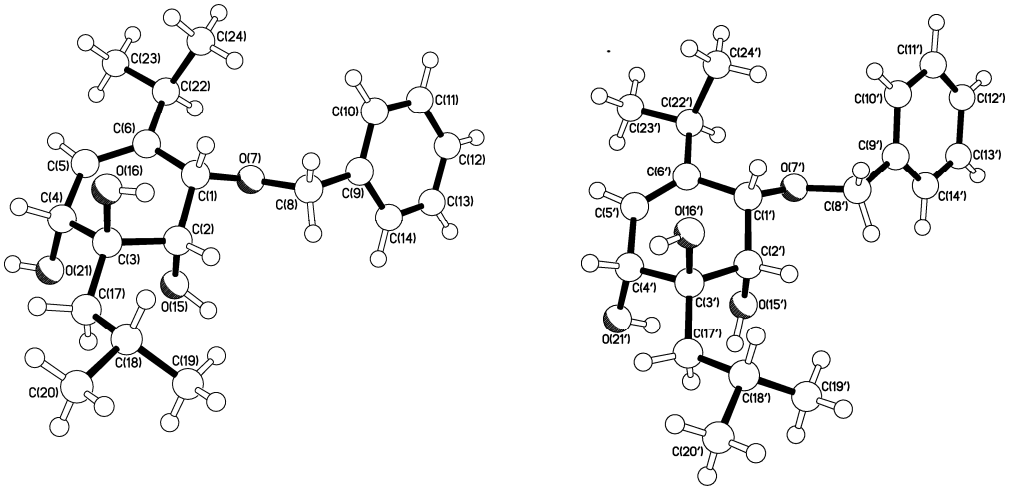


Table 1. Crystal data and structure refinement for **138**.

Table 1. Crystal data and structure refinement for **138.**

Identification code	PG0801
Empirical formula	C20 H30 O4
Formula weight	334.44
Temperature	173(2) K
Diffractometer, wavelength	OD Xcalibur PX Ultra, 1.54184 Å
Crystal system, space group	Monoclinic, P2(1)/c
Unit cell dimensions	a = 14.5758(3) Å α = 90°
	b = 9.37699(17) Å β = 101.251(3)°
	c = 28.4197(9) Å γ = 90°
Volume, Z	3809.67(16) $Å^3$, 8
Density (calculated)	1.166 Mg/m^3
Absorption coefficient	0.637 mm^{-1}
F(000)	1456
Crystal colour / morphology	Colourless platy needles
Crystal size	0.31 x 0.24 x 0.01 mm^3

θ range for data collection	3.09 to 73.27°
Index ranges	−16<=h<=17, −11<=k<=11, −35<=l<=35
Reflns collected / unique	51329 / 51329 [R(int) = 0.0000]
Reflns observed [F>4σ(F)]	22052
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	51329 / 0 / 448
Goodness-of-fit on F^2	1.022
Final R indices [F>4σ(F)]	R1 = 0.1157, wR2 = 0.3357
R indices (all data)	R1 = 0.1869, wR2 = 0.3561
Largest diff. peak, hole	0.738, −0.694 eÅ^{-3}
Mean and maximum shift/error	0.000 and 0.001

Table 2. Bond lengths [Å] and angles [°] for **138**.

C(1)-O(7)	1.448(3)	C(11)-C(12)	1.382(5)
C(1)-C(6)	1.496(4)	C(12)-C(13)	1.342(5)
C(1)-C(2)	1.518(4)	C(13)-C(14)	1.387(4)
C(2)-O(15)	1.435(3)	C(17)-C(18)	1.512(4)
C(2)-C(3)	1.558(3)	C(18)-C(20)	1.542(4)
C(3)-O(16)	1.452(3)	C(18)-C(19)	1.545(4)
C(3)-C(17)	1.500(4)	C(22)-C(24)	1.530(4)
C(3)-C(4)	1.552(3)	C(22)-C(23)	1.531(4)
C(4)-O(21)	1.453(3)	C(1')-O(7')	1.432(3)
C(4)-C(5)	1.481(4)	C(1')-C(2')	1.493(4)
C(5)-C(6)	1.380(4)	C(1')-C(6')	1.520(4)
C(6)-C(22)	1.510(4)	C(2')-O(15')	1.451(3)
O(7)-C(8)	1.424(3)	C(2')-C(3')	1.530(4)
C(8)-C(9)	1.497(4)	C(3')-O(16')	1.460(3)
C(9)-C(10)	1.381(4)	C(3')-C(17')	1.514(4)
C(9)-C(14)	1.404(4)	C(3')-C(4')	1.555(4)
C(10)-C(11)	1.400(5)	C(4')-O(21')	1.462(3)

C(4')-C(5')	1.506(4)	C(10)-C(9)-C(8)	122.2(3)
C(5')-C(6')	1.319(4)	C(14)-C(9)-C(8)	118.9(3)
C(6')-C(22')	1.506(4)	C(9)-C(10)-C(11)	119.9(3)
O(7')-C(8')	1.447(3)	C(12)-C(11)-C(10)	120.2(4)
C(8')-C(9')	1.503(4)	C(13)-C(12)-C(11)	119.8(3)
C(9')-C(10')	1.370(4)	C(12)-C(13)-C(14)	121.7(3)
C(9')-C(14')	1.374(4)	C(13)-C(14)-C(9)	119.5(3)
C(10')-C(11')	1.391(5)	C(3)-C(17)-C(18)	118.1(2)
C(11')-C(12')	1.392(6)	C(17)-C(18)-C(20)	110.1(3)
C(12')-C(13')	1.353(5)	C(17)-C(18)-C(19)	113.5(2)
C(13')-C(14')	1.390(5)	C(20)-C(18)-C(19)	108.2(3)
C(17')-C(18')	1.542(4)	C(6)-C(22)-C(24)	110.9(2)
C(18')-C(19')	1.498(5)	C(6)-C(22)-C(23)	115.1(3)
C(18')-C(20')	1.523(4)	C(24)-C(22)-C(23)	110.5(3)
C(22')-C(23')	1.523(4)	O(7')-C(1')-C(2')	110.8(2)
C(22')-C(24')	1.544(4)	O(7')-C(1')-C(6')	107.3(2)
O(7)-C(1)-C(6)	107.0(2)	C(2')-C(1')-C(6')	113.8(2)
O(7)-C(1)-C(2)	109.6(2)	O(15')-C(2')-C(1')	110.0(2)
C(6)-C(1)-C(2)	114.9(2)	O(15')-C(2')-C(3')	104.9(2)
O(15)-C(2)-C(1)	111.4(2)	C(1')-C(2')-C(3')	113.5(2)
O(15)-C(2)-C(3)	104.9(2)	O(16')-C(3')-C(17')	111.9(2)
C(1)-C(2)-C(3)	111.8(2)	O(16')-C(3')-C(2')	103.8(2)
O(16)-C(3)-C(17)	110.6(2)	C(17')-C(3')-C(2')	115.8(2)
O(16)-C(3)-C(4)	105.7(2)	O(16')-C(3')-C(4')	105.7(2)
C(17)-C(3)-C(4)	113.5(2)	C(17')-C(3')-C(4')	111.8(2)
O(16)-C(3)-C(2)	104.2(2)	C(2')-C(3')-C(4')	107.0(2)
C(17)-C(3)-C(2)	115.0(2)	O(21')-C(4')-C(5')	109.1(2)
C(4)-C(3)-C(2)	107.0(2)	O(21')-C(4')-C(3')	110.6(2)
O(21)-C(4)-C(5)	110.2(2)	C(5')-C(4')-C(3')	114.9(2)
O(21)-C(4)-C(3)	110.2(2)	C(6')-C(5')-C(4')	124.9(3)
C(5)-C(4)-C(3)	115.2(2)	C(5')-C(6')-C(22')	123.7(3)
C(6)-C(5)-C(4)	124.0(3)	C(5')-C(6')-C(1')	119.4(3)
C(5)-C(6)-C(1)	119.2(3)	C(22')-C(6')-C(1')	116.9(2)
C(5)-C(6)-C(22)	122.6(3)	C(1')-O(7')-C(8')	113.28(19)
C(1)-C(6)-C(22)	118.0(2)	O(7')-C(8')-C(9')	109.0(2)
C(8)-O(7)-C(1)	113.83(19)	C(10')-C(9')-C(14')	120.6(3)
O(7)-C(8)-C(9)	108.9(2)	C(10')-C(9')-C(8')	118.4(3)
C(10)-C(9)-C(14)	118.9(3)	C(14')-C(9')-C(8')	121.0(3)

C(9')-C(10')-C(11')	119.3(3)
C(10')-C(11')-C(12')	119.6(4)
C(13')-C(12')-C(11')	120.7(3)
C(12')-C(13')-C(14')	119.6(3)
C(9')-C(14')-C(13')	120.2(3)
C(3')-C(17')-C(18')	116.8(2)
C(19')-C(18')-C(20')	108.5(3)
C(19')-C(18')-C(17')	113.1(3)
C(20')-C(18')-C(17')	109.4(3)
C(6')-C(22')-C(23')	114.4(3)
C(6')-C(22')-C(24')	111.9(3)
C(23')-C(22')-C(24')	110.2(3)

Appendix C: Experimental for Biological Assays

$^{45}Ca^{2+}$ Flux Assay (Geert Bultnyck)

L15 cells were obtained by stable exogenous expression of IP_3R1 in L fibroblasts. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, 3.8 mM L-glutamine, 0.9% (v/v) non-essential amino acids, 85 IU/mL penicillin, 85 µg/mL streptomycin, and 20 mM HEPES, pH 7.4. $^{45}Ca^{2+}$ fluxes were performed on saponin-permeabilized cells. The cells were seeded in 12-well clusters (*Costar*, Cambridge, MA) at a density of approximately 4 x 10^4 cm^2. Experiments were carried out on confluent monolayers of cells at the seventh day after plating. The cells were permeabilized by incubating them for 10 min with a solution containing 120 mM KCl, 30 mM imidazole hydrochloride, pH 6.8, 2 mM $MgCl_2$, 1 mM ATP, 1 mM EGTA, and 40 µg/mL saponin at 30 °C. The non-mitochondrial Ca^{2+} stores were loaded for 45 min at 30 °C in 120 mM KCl, 30 mM imidazole hydrochloride, pH 6.8, 5 mM $MgCl_2$, 5 mM ATP, 0.44 mM EGTA, 10 mM NaN_3, and 150 nM free $^{45}Ca^{2+}$ (28 µCi/mL). The cells were then washed twice with 1 mL of efflux medium containing 120 mM KCl, 30 mM imidazole hydrochloride, pH 6.8, 1 mM EGTA, and 10 µM thapsigargin. The efflux medium was replaced every 2 min, and the efflux was performed at 30 °C. At the end of the experiment, the $^{45}Ca^{2+}$ remaining in the stores was released by incubation with 1 mL of a 2 % sodium dodecyl sulfate solution for 30 min. Ca^{2+} release is plotted as the fractional loss (*i.e.*, the amount of Ca^{2+} released in 2 min divided by the total store Ca^{2+} content at that time). The latter value was calculated by summing in retrograde order the amount of tracer remaining in the cells at the end of the efflux and the amounts of tracer collected during the successive time intervals. The Ca^{2+} release provoked by IP_3 and 4-*C*-methyl IP_3 was normalized to the maximal releasable Ca^{2+}, measured by the addition of 10 µM A23187.

Synaptojanin Assays (Jessica Knott)

Malachite Green Assay:

Compounds, enzyme (SAL) and Tris (50mM) were incubated at room temperature for 15 min.

$MgCl_2$ was then added to make a final concentration of 4mM.

Boiled enzyme was used as a negative control.

The final volume of the assay was 80 uL

IP_3 was used to start the reaction and samples were incubated for 25 min at 37 ^oC.

To stop the enzyme reaction, equi-volume of the acidic malachite green dye was added

The mixture was allowed to develop for 10 min

An elisa reader was used to read the absorption of the dye at a wavelength of 625 nm

OMFP assay

The total volume of the assay was 160uL

Inhibitor (100uM), Tris (50mM) and enzyme were incubated in a 96 well plate for 15 minutes.

MgCl2 (4mM) was then added

OMFP (300uM) was added to start the reaction

The well plate was incubated at 37degC

The control consisted of the same as above but without enzyme

The formation of o-methylfluorescein from OMFP was detected by fluorescence at excitation = 485nm and emission 525nm at 5 minute intervals up to 40 minutes

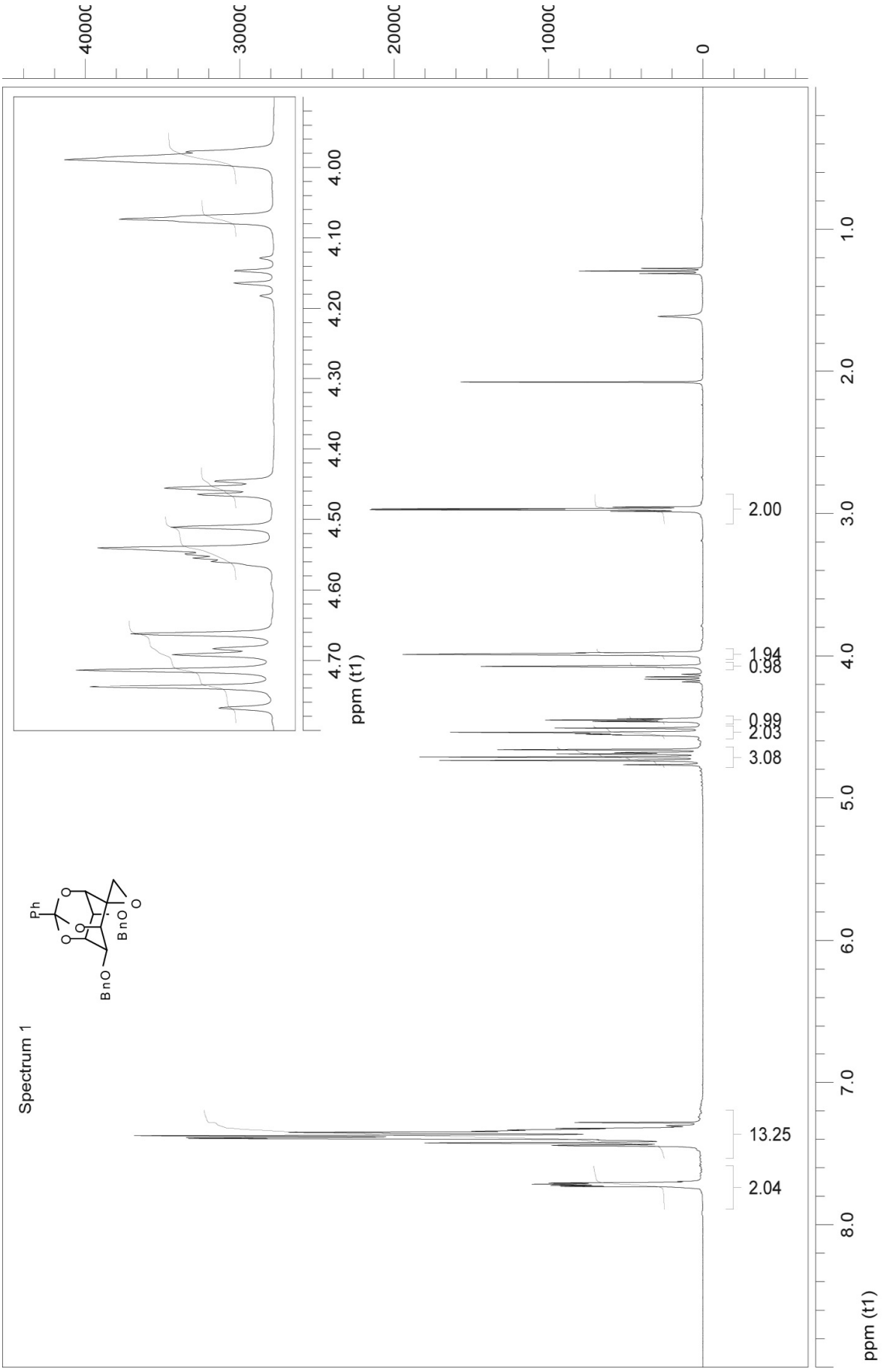
The activites in the graph (Figure 5.25) were taken at 35 minutes

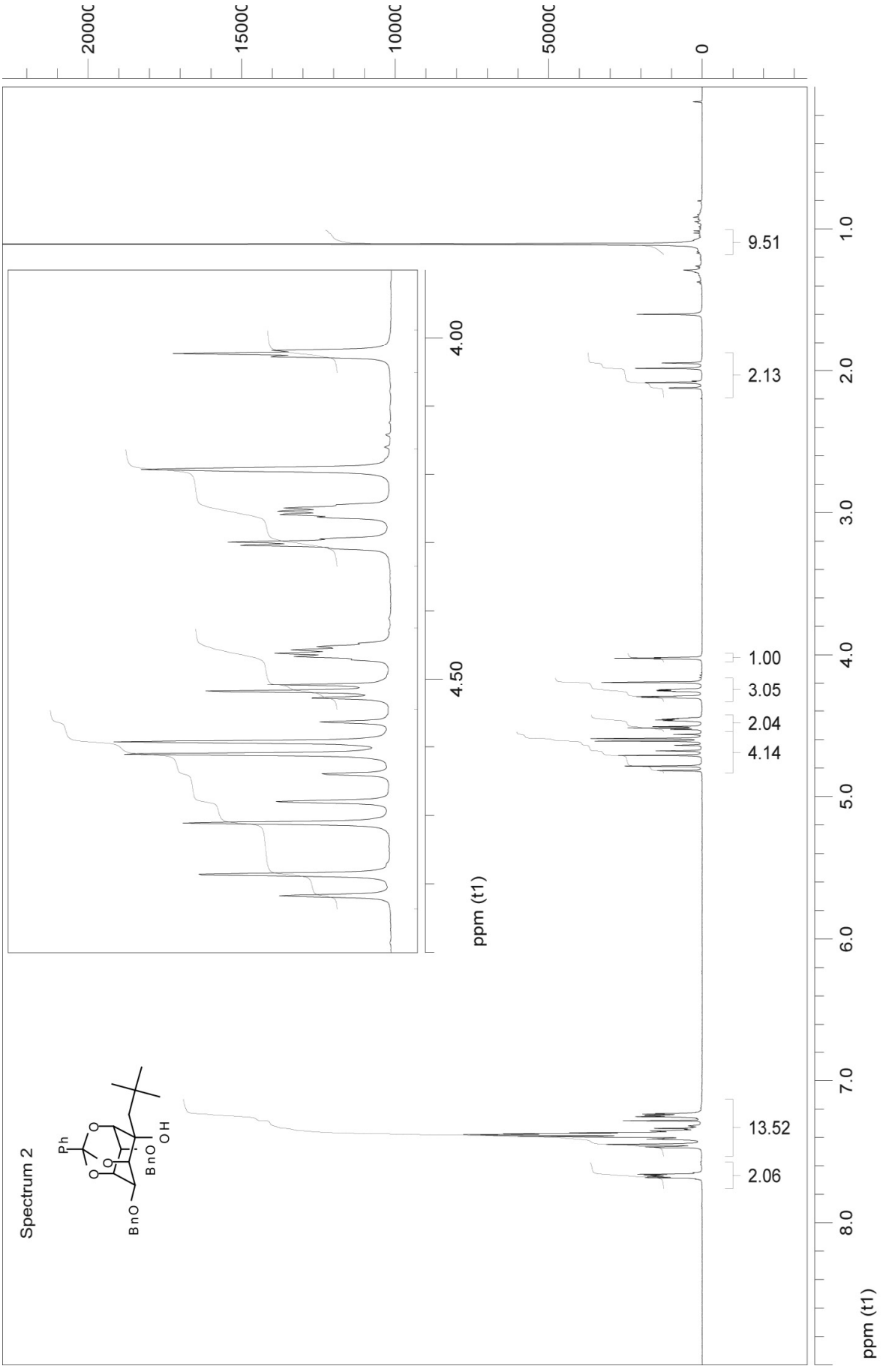
Appendix D: NMR spectra

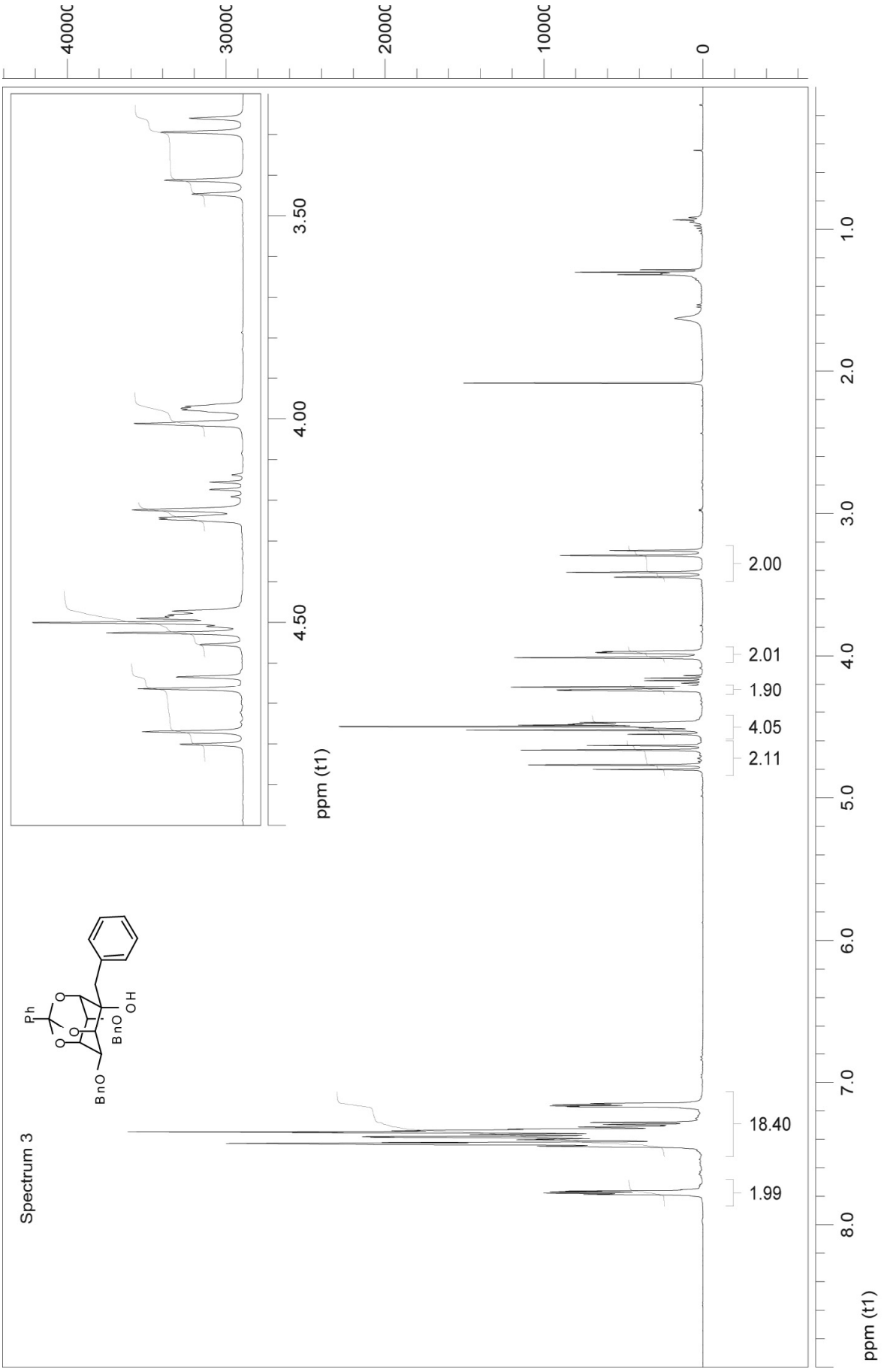
The spectra in this thesis were measured on 270 MHz, 400 MHz and 500 MHz machines, depending on availability. There is a noticeable difference in quality of spectra and therefore possible interpretation, depending on the machine used.

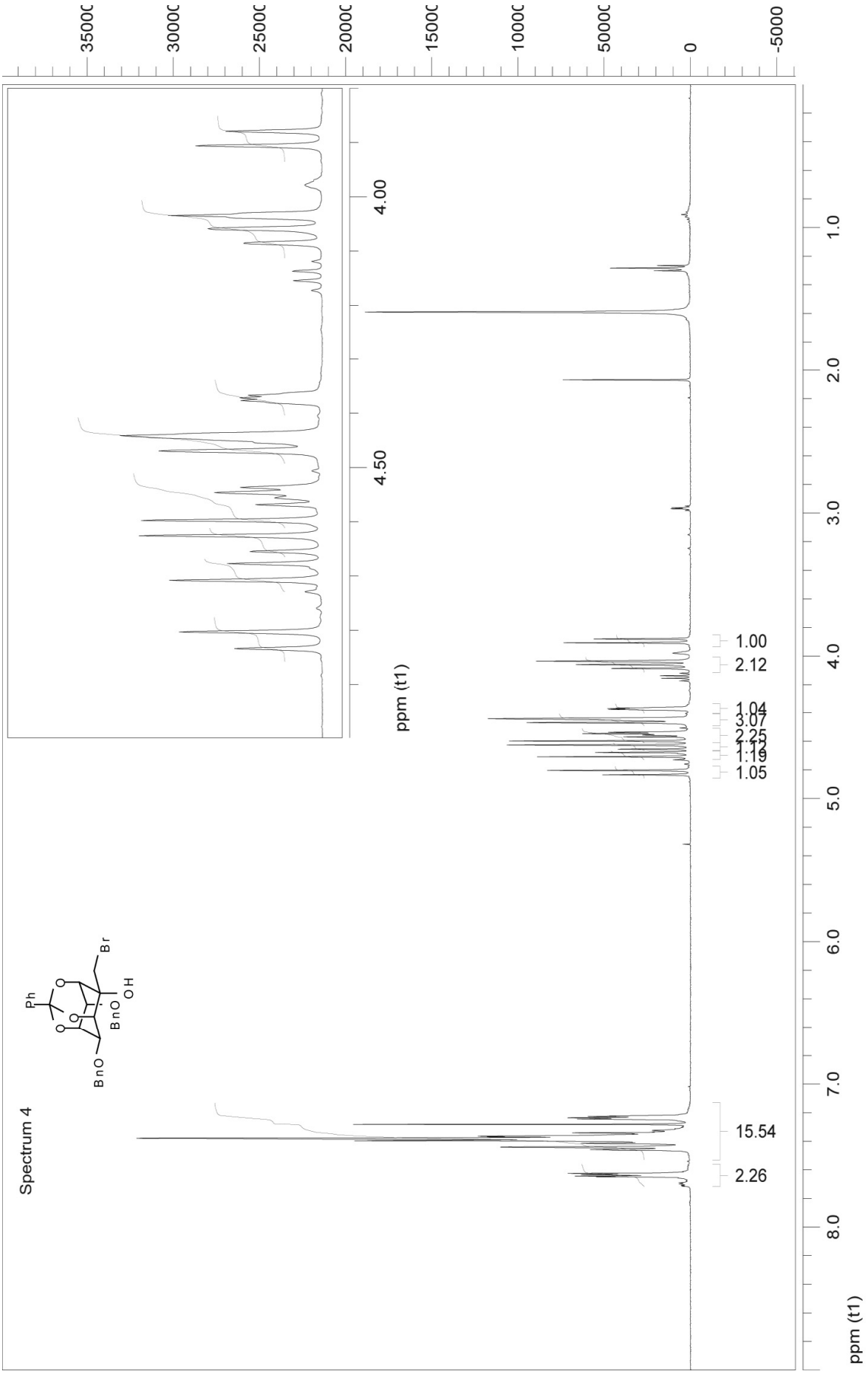
Ten spectra are included here to illustrate the characteristic inositol signals at each stage of the synthesis

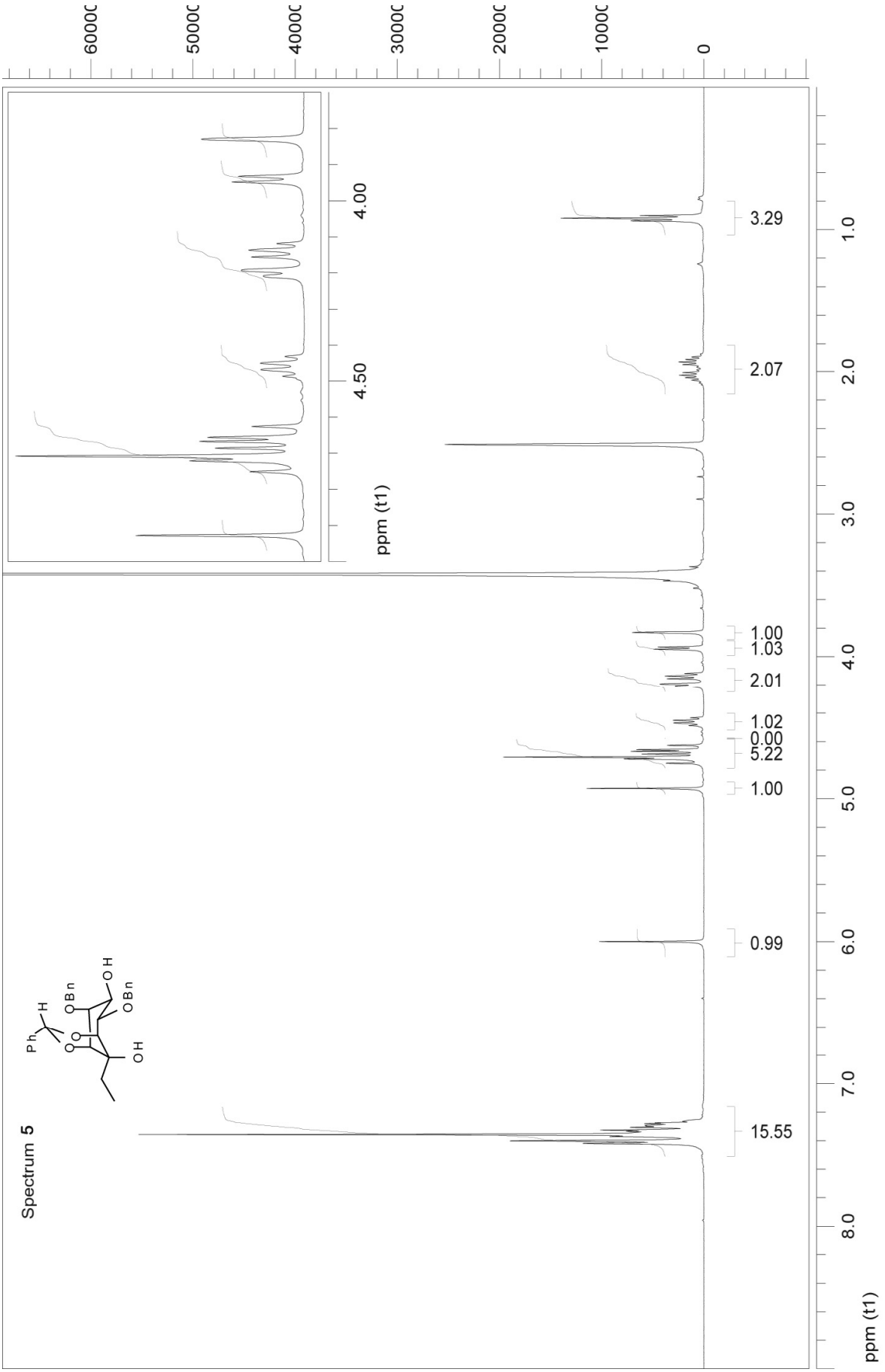
1. 2,6-*O*-Dibenzyl-4,4-*O,C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate, **98**
2. 2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol 1,3,5-*O*-orthobenzoate, **144**
3. 2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate, **125**
4. 2,6-*O*-Dibenzyl-4-*C*-bromomethyl-*myo*-inositol 1,3,5-*O*-orthobenzoate, **126**
5. 2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol 3,5-*O*-benzylidene acetal, **176b**
6. 2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol, **151f**
7. 1,5-*O*-Diacetyl-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol, **177f**
8. 4-*C*-Methyl IP_4, **191a**
9. 4-*C*-Ethyl IP_4, **191b**
10. 4-*C*-Benzyl IP_3, **203**

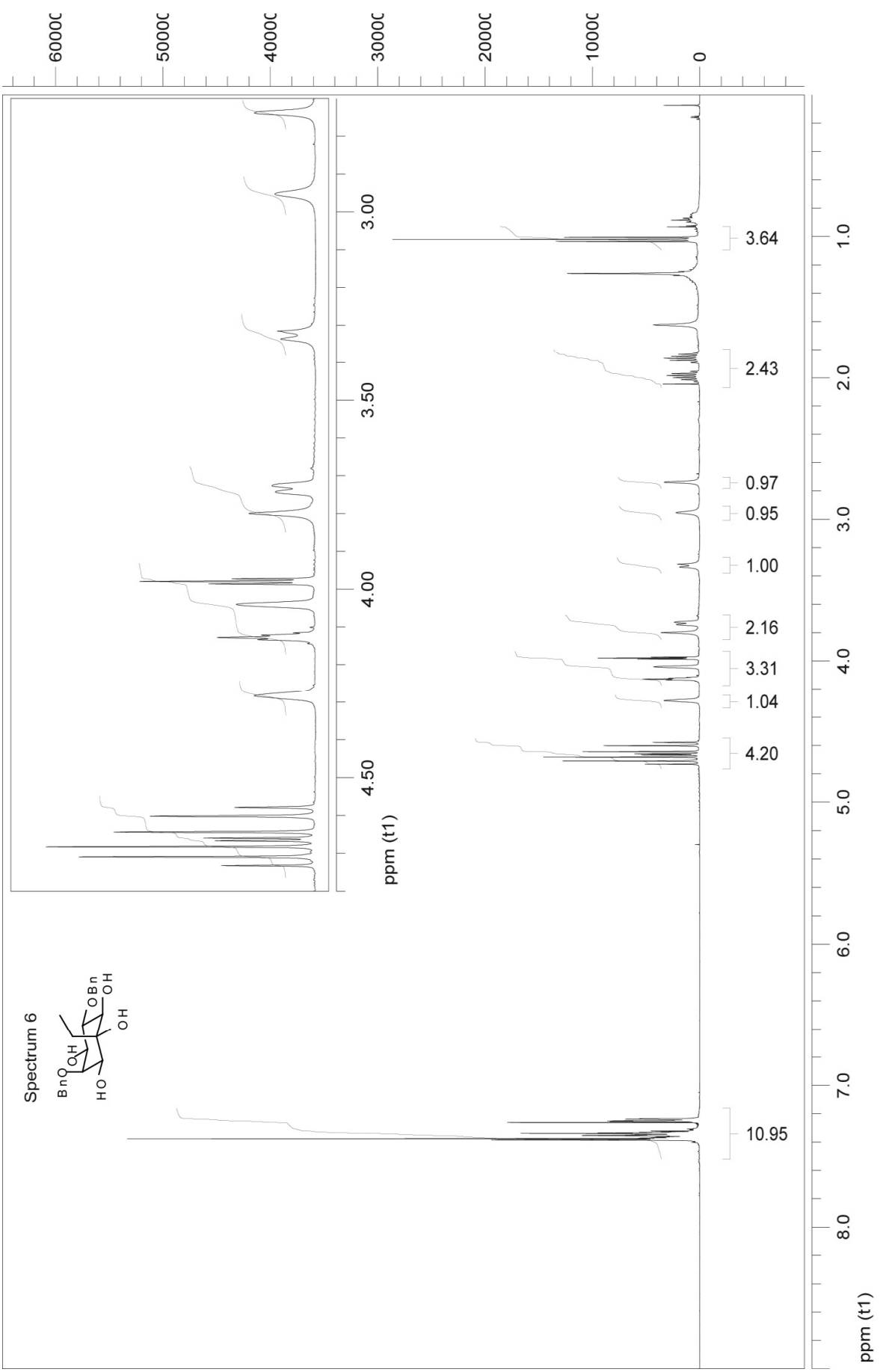

Spectrum 1
Ph
BnO
BnO
O
2.00
1.94
0.98
0.99
2.03
3.08
13.25
2.04
ppm (t1)

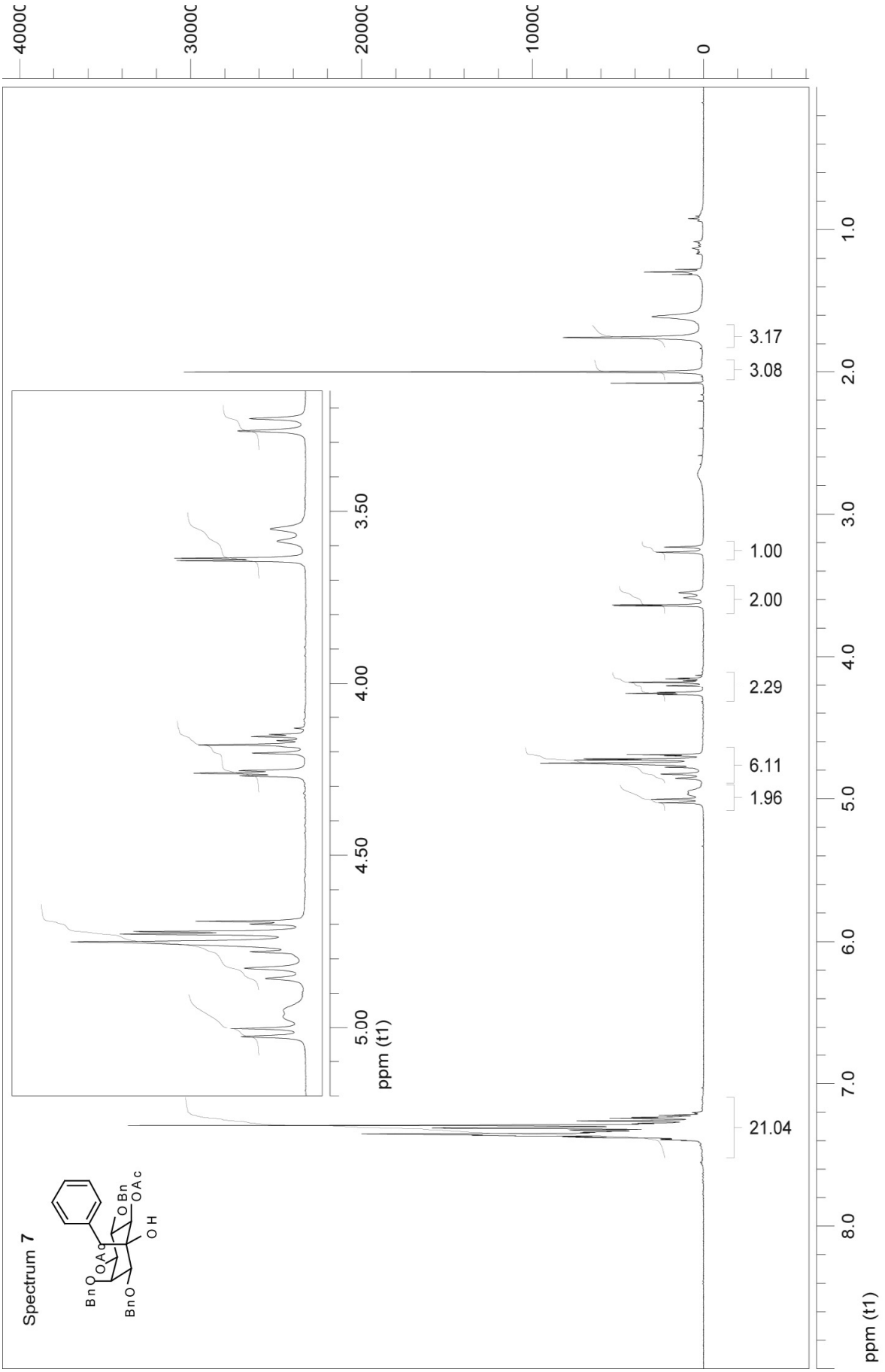

Spectrum 2
Ph
BnO
BnO
OH
9.51
2.13
1.00
3.05
2.04
4.14
13.52
2.06
4.00
4.50
ppm (t1)
ppm (t1)

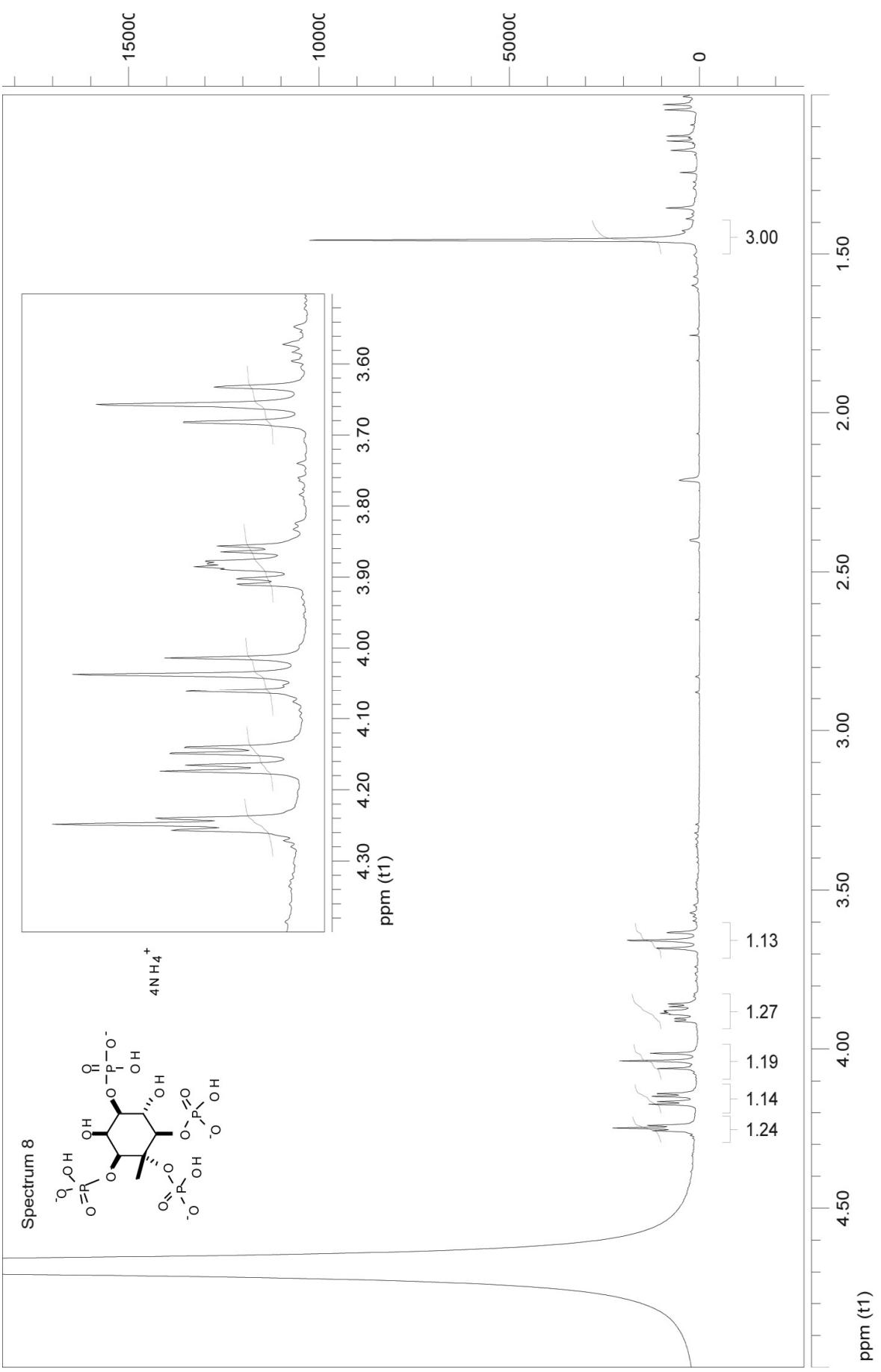

Spectrum 3
Ph
BnO
OH
BnO
ppm (t1)
2.00
2.01
1.90
4.05
2.11
18.40
1.99

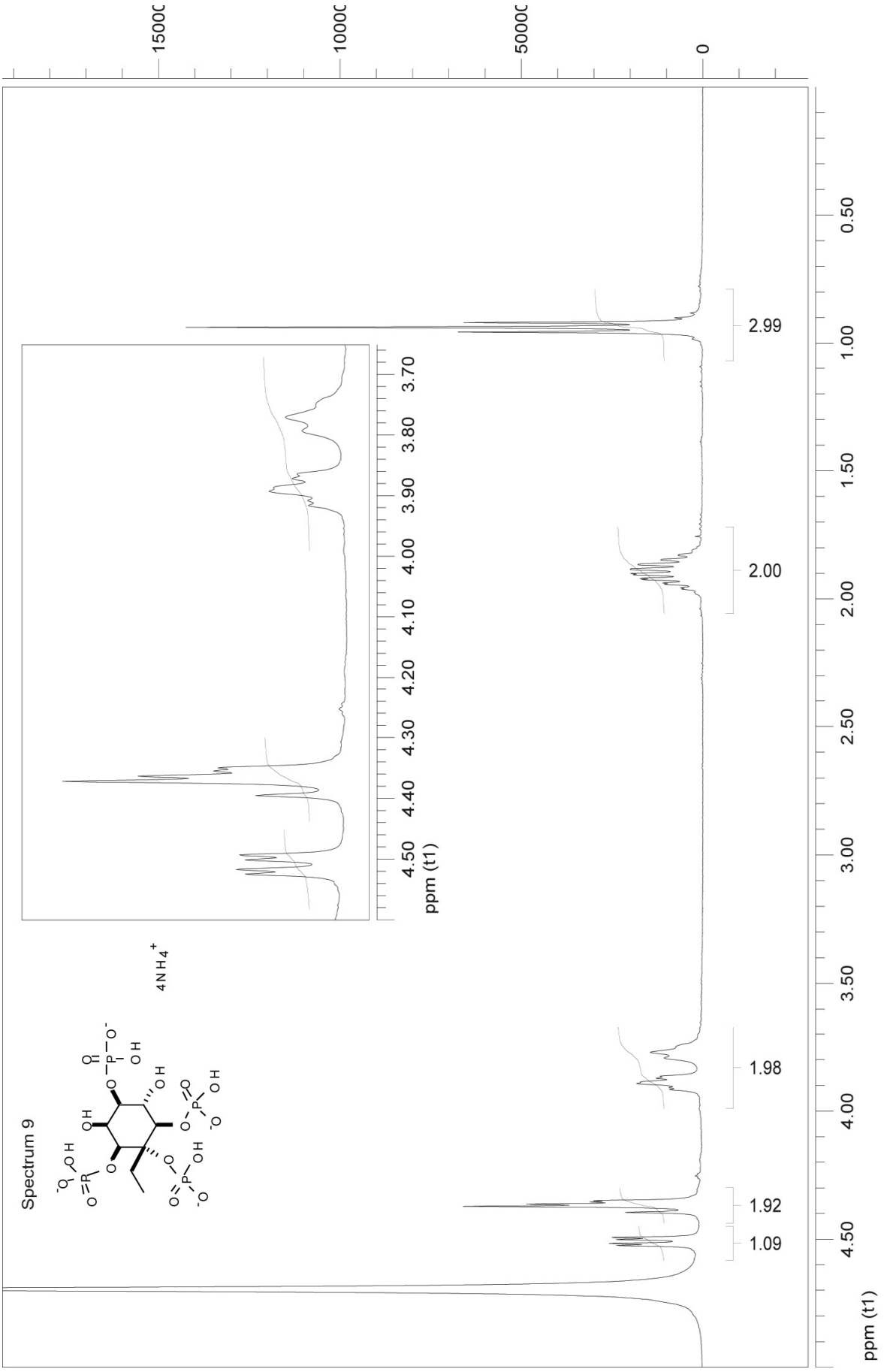

Spectrum 4
Ph
BnO
BnO
OH
Br
4.00
4.50
ppm (t1)
1.00
2.12
1.04
3.07
2.25
1.19
1.05
15.54
2.26
ppm (t1)

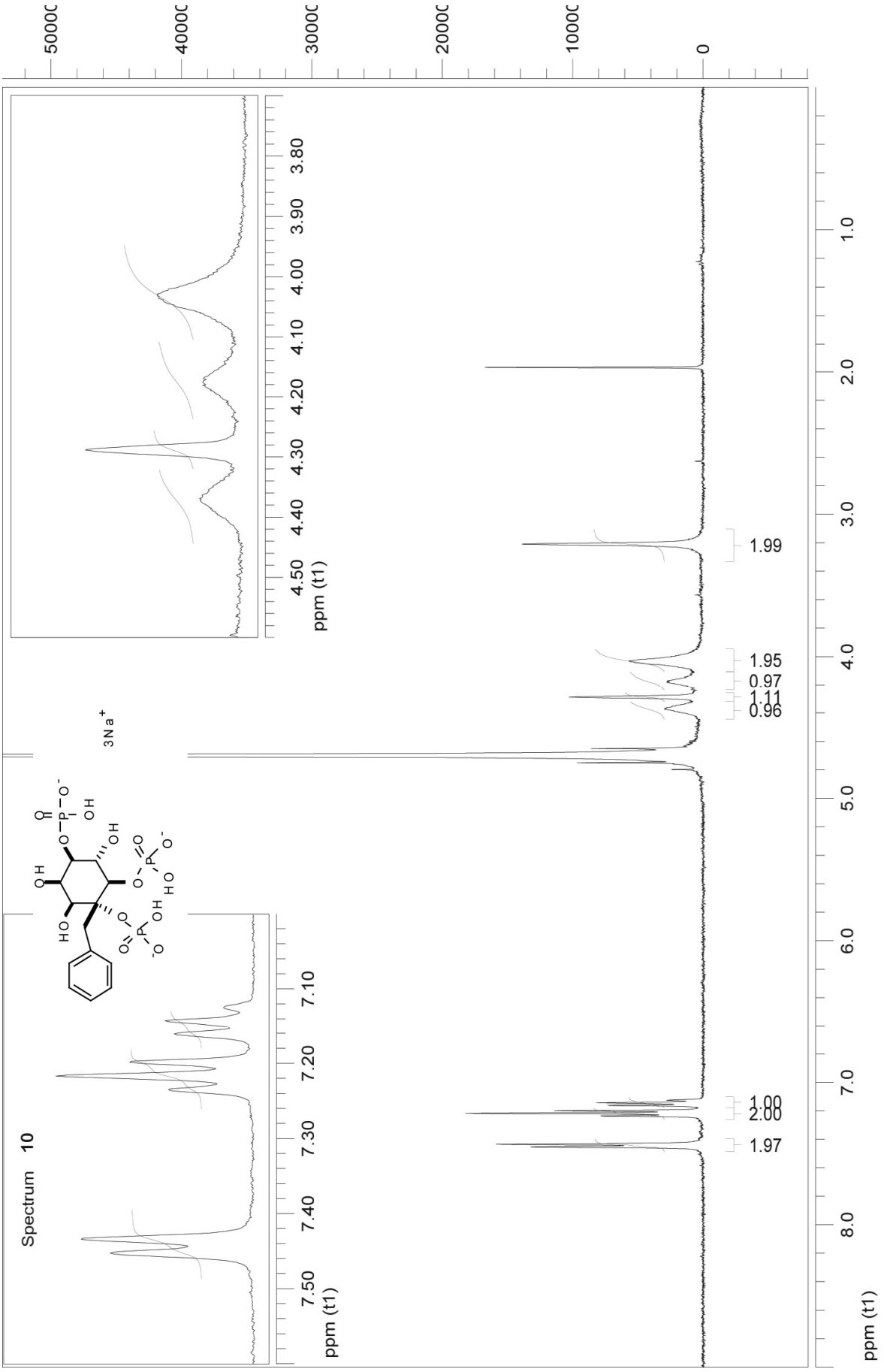

Spectrum 5
Ph
H
O
OBn
OBn
OH
OH
3.29
2.07
1.00
1.03
2.01
1.02
0.00
5.22
1.00
0.99
15.55
4.00
4.50
ppm (t1)
ppm (t1)


Spectrum 6
3.64
2.43
0.97
0.95
1.00
2.16
3.31
1.04
4.20
10.95
ppm (t1)


Spectrum 7
3.17
3.08
1.00
2.00
2.29
6.11
1.96
21.04
ppm (t1)


Spectrum 8
4 N H4+
3.00
1.13
1.27
1.19
1.14
1.24
ppm (t1)


Spectrum 9
4NH4+
ppm (t1)
2.99
2.00
1.98
1.92
1.09


Spectrum 10
3Na+
1.99
1.95
0.97
1.11
0.96
1.00
2.00
1.97
ppm (t1)

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